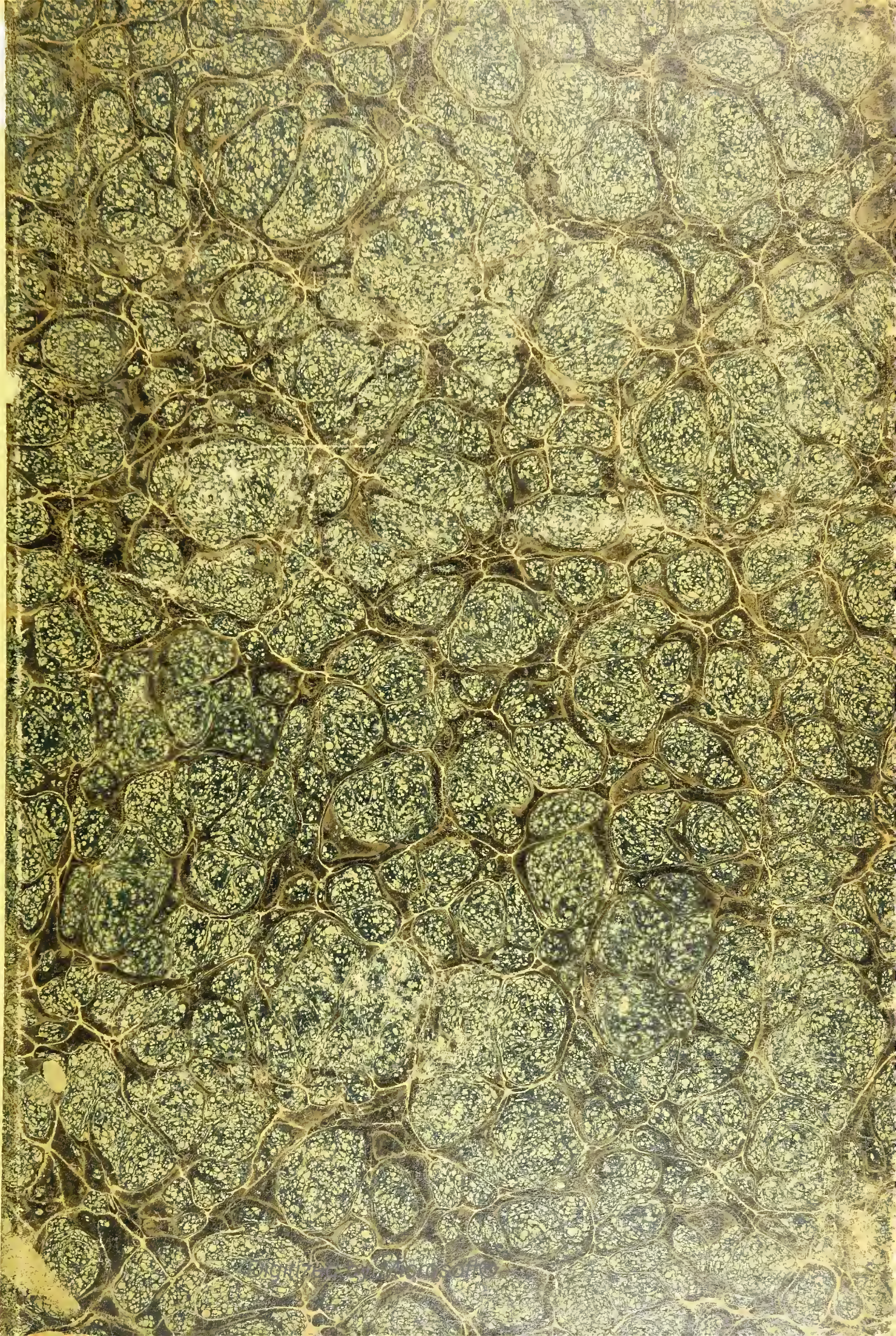


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[PLATES 7-10.]

THE ŒSTROUS CYCLE AND THE FORMATION OF THE  
CORPUS LUTEUM IN THE SHEEP.

BY

FRANCIS H. A. MARSHALL, B.A.

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III.—*The Œstrous Cycle and the Formation of the Corpus Luteum in the Sheep.*

*By* FRANCIS H. A. MARSHALL, *B.A.*

*Communicated by* Professor J. C. EWART, *F.R.S.*

Received February 17,—Read March 12, 1903.

[PLATES 7–10.]

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*Introduction.*

MY investigations on the changes undergone by the genital organs, and more particularly the uterus, on ovulation and its relation to œstrus and coition, and on the mode of development of the corpus luteum in the sheep, were commenced in the summer of 1900, and continued until the close of the sheep's sexual season in the

within the species, whereas the period of gestation does not proportionately vary, Dr. BEARD'S hypothesis (1897) that the time of birth is regulated by the approaching termination of an ovulation interval, can scarcely be maintained.\* In this connection it is interesting to note that Mr. HEAPE (1900) finds that the length of the diœstrous cycle in the rabbit may vary by as much as ten or eleven days.†

I have shown above from my own observations that the Scotch Black-faced sheep when kept in the Lowlands of Scotland may have at least three recurrent periods of œstrus in the absence of the ram, and flockmasters inform me that under very favourable conditions there may be five or six. Mr. HEAPE (1900), on the authority of Mr. CAMERON states that Scotch Black-faced sheep have only two recurrent periods of œstrus in each sexual season.‡ Mr. HEAPE further informs me that Mr. CAMERON'S observations refer to hill sheep in the Highlands, and that the sexual season for these animals is, collectively, six weeks; that is, it consists of two recurrent diœstrous cycles, each of three weeks duration. Mr. HEAPE also informs me that Mr. CAMERON states that the period of œstrus with the Highland Black-faced ewes occupies five days.

It can hardly be doubted that of the two conditions of the Scotch Black-faced ewes that of the Highland sheep is the more natural one,§ for the sheep is essentially a mountain animal in its wild state, being almost entirely confined to mountain districts in the Holarctic region, and only just getting into the more tropical Oriental region. "The immense mountain ranges of Central Asia, the Pamir, and Thian-Shan of Turkestan may be looked upon as the centre of their habitat" (FLOWER and LYDEKKER, 1891).

What little is known about the breeding habits of wild sheep points to the conclusion that at least some species are monœstrous, or at most diœstrous. The accounts given by LYDEKKER (1898) of the rutting of *Ovis musimon*, *O. vignei*, *O. ammon*, and *O. canadensis* show that these sheep in their wild state have a definite annual sexual season, which from its short duration renders it probable that they are monœstrous. Thus the sexual season in *Ovis canadensis* (var. *typica*) is described as

\* According to BEARD, just under ten ovulation intervals go to the sheep's gestation.

† An experienced breeder of Angus and Shorthorn cattle in Banffshire, in answer to a query regarding the length of the diœstrous cycle in cows of those breeds, states that this interval varies from two to three weeks.

‡ SCOTT, writing on "The Practice of Sheep Farming," states that with hill sheep generally, the "tupping season" (*i.e.*, the sexual season) begins about November 22, and terminates with the close of the year, and that the lambing season begins about April 17 and ends about May 26. These facts render it improbable that there are usually more than two diœstrous cycles with hill sheep, but it must be remembered that, if there are a sufficient number of rams to a flock of a given size, the ewes usually succeed in getting served at an early œstrus.

§ Professor EWART informs me that the duration of the sexual season (or the number of diœstrous cycles in a season) with horses varies according to the character of the breed. Thus, in semi-wild ponies (*e.g.*, Iceland or Exmoor ponies) the sexual season terminates with the approach of autumn. In the more domestic types of horses, œstrus may continue to recur for a far longer period.

being about the second week of November. Similarly it may be inferred from PRJEVALSKY'S accounts (1876) that *Ovis poli*, *O. burrhel*, and *O. argali* are monœstrous, and breed once a year.

BLANFORD'S notes (1891) on the rutting habits of the Indian species point in the same direction, for it must be remembered that the accounts given of the rutting periods invariably apply to the sheep collectively in particular areas, and that it is improbable that all the ewes in these areas come in season at precisely the same time. BLANFORD'S descriptions show further that the rutting periods of some of the wild species, like those of many domestic breeds, may vary with the locality, for while with *Ovis vignei* in the Panjáb the sexual season is September, with the same species in Astor it must be very considerably later, since the young in the latter area are produced about the beginning of June. (See also LYDEKKER, 1898.)

The Barbary wild sheep (*Ovis tragelaphus*) in the Zoological Society's Gardens, Regent's Park, is said to be monœstrous, breeding once annually (HEAPE, 1900). The same is stated to be the case with *Ovis burrhel* in the Gardens, while the Mouflon (*Ovis musimon*) in captivity may experience two or more recurrent œstrous periods during its annual sexual season.\*

Scotch Highland sheep, as already stated, are dioestrous, whereas sheep of the same breed in the Lowlands resemble the great majority of the domestic breeds in being polyœstrous with a single sexual season annually. Of other British breeds, Dorset Horns possess the characteristic of being able to produce two crops of lambs within a year, but this is discouraged by the sheep breeders, as it tends to deteriorate the ewes. Hampshire Down sheep also are stated to be sometimes able to breed twice annually.

The same is not unfrequently the case with ewes belonging to the Limestone breed of some districts of Westmoreland and Derbyshire. This breed, although it has never come into prominence, has existed for many years as an important local variety, and is described by Professor WALLACE (1893). Mr. ROWLAND PARKER, of Moss End, Malathorpe, Westmoreland, from whom Professor WALLACE obtained information, has been good enough to inform me further regarding the sexual and lambing seasons of Limestone sheep. The general lambing season is from the middle of February to the middle of March, but lambs are frequently born considerably earlier. Mr. PARKER says that he has known the ewes take the ram very early when suckling their lambs, a second crop being born in August, but when this occurred the ewes did not breed the year after. Such an increase in the duration of the sexual season is the more remarkable as occurring in north-country sheep classed as belonging to a

\* These statements are from seemingly reliable information given to me by keepers in the Zoological Society's Gardens, London. Subsequently Mr. F. E. BEDDARD, F.R.S., Prosector to the Zoological Society, has very kindly obtained further information, confirming the statement that the Barbary and Burrhel sheep in the Gardens are monœstrous. The Barbary sheep are described as rutting about October, and the Burrhel sheep in January or February.

mountain breed, and which, although thriving well and attaining their maximum size on dry lowland pasture, are hardy and active, living naturally on dry heaths or bare mountain pastures (WALLACE, 1893).

Although most foreign sheep resemble the majority of British breeds in showing varying degrees of polyœstrum, but a single sexual season annually, ARISTOTLE long ago recorded the fact that "in some places where the weather is warm and fine, and food is abundant," sheep may have young twice a year. When this is the case the spring sexual season should probably be regarded as of the nature of a continuation of a series of diœstrous cycles which started at the previous autumn sexual season, but were interrupted by gestation. A breeder of Dorset Horn sheep in the Isle of Wight informs me that in the absence of the ram œstrus may keep on recurring at regular intervals in the ewes from the autumn sexual season (during which they are normally allowed to become pregnant) onwards until the following spring. It would appear, however, that in these sheep there is a short anœstrous period (in the absence of gestation) during part of the summer, but that its length is a variable quantity, depending upon whether or not there has been a winter gestation, and being further complicated by such influences as lactation, food supply, and climatic conditions.

Just as Dorset Horn ewes will come in season again within a short period of lambing, so sheep belonging to other breeds will often experience œstrus after an early abortion. I have had two cases of Scotch Black-faced ewes experiencing œstrus early in February. This is a great deal later than the ordinary sexual season, which for this breed usually begins late in October or early in November. Unfortunately the sheep in question had not previously been under proper observation, but I think it not unlikely that they had become pregnant to a ram at an early œstrus (before they came into my possession), and subsequently aborted.\*

Such cases as these further justify the view that where sheep can have lambs twice a year, the œstri of the additional sexual season are comparable to the œstri occurring after early abortions in those sheep which can produce lambs only once annually, the full series of diœstrous cycles characteristic of the respective breeds being interrupted by the whole or part of a gestation.

Mr. NELSON ANNANDALE tells me, from information given him by the RAJAH OF JALOR, that the horned sheep which run half wild in the old kingdom of Patani, in the Malay Peninsula, normally have lambs twice every year. Mr. ANNANDALE himself saw lambs apparently a few weeks old in April and also in December.

The Merino sheep, which is almost cosmopolitan, although split up into many sub-

\* Mr. IRVING DENT, of Ravensneuk, Penycuik, from whom I obtained many of my sheep, informs me of a case of a Scotch Black-faced ewe in his possession, which was in season in November and again in the following April, giving birth to a lamb in September. The animal had become pregnant at the normal time, and aborted the embryo. The case is interesting, showing that a sheep belonging to a mountain breed in Scotland can, very exceptionally, come "in use" and ovulate many months after the termination of the regular sexual season. The same ewe again came "in use" at the commencement of the next sexual season, or at the end of the following October or beginning of November.

breeds in different places, shows considerable variation in sexual activity in different parts of the world. BONNET (1884) describes the "brunst" as returning seven months after parturition; but he made no observations himself on the "brunst" periods, which, as was the case with my sheep, are said to last for a short period, and sometimes for only a few hours.

Among the Merino sheep of Cape Colony the general lambing season is September (the spring month, corresponding to March in this country). But lambs are frequently born earlier or during winter. At high altitudes, however, where the ewes subsist entirely upon the natural produce of the veldt, the lambing season is stated to be October, from which it may be inferred that the sexual season also begins a month later than the usual time over Cape Colony. On the other hand, in the low country, below the second range of mountains, there are two seasons for lambing, the autumn season being in April and May. But here the lambing periods are limited, since the ewes do not usually come in season until about the middle of December (WALLACE, 1896).

Probably the maximum amount of sexual activity reached by any sheep is that attained to by the Merinos of some parts of Australia, which are described as being able to breed all the year round, a fact which implies, in the absence of gestation, an almost continuous series of dioestrous cycles. The report of the Chief Inspector of Stock for New South Wales divides the time of lambing into six periods, which embrace the whole year (WALLACE, 1891).

There is, then, a complete gradation from the almost certainly monœstrous condition of at least some wild species of sheep, in which there is a single sexual season annually, down to the extreme of polyœstrum reached by the Merinos of New South Wales, which are said to be able to breed throughout the whole year. The instances cited above have been chosen mainly to illustrate this gradation, to which I have not seen attention called hitherto.\*

That the variability is dependent largely upon climate and food supply can hardly be doubted, and the Black-faced sheep and the Merinos in different parts of Scotland and Cape Colony respectively furnish direct evidence that this is the case. On the other hand, there can be no doubt that the varying degrees of breeding activity are also in part racial characteristics, as is shown, for example, by the Dorset Horn sheep of the south of England, and more evidently by the Limestone sheep of Westmoreland and Derbyshire. But that an increase in the duration (or more frequent recurrence) of the sexual season is not necessarily a highly artificial condition or the part result of special attention in regard to food supply, &c., on the part of the flockmaster, is shown by such a condition occurring normally among the half-wild sheep of Patani.

\* From HEAPE'S notes (1900) on the subject it would seem possible that goats and pigs could be made to illustrate a similar gradation from the monœstrous habit in the natural state to varying degrees of polyœstrum, under domestication or in captivity, but the facts about these animals are less perfectly known.

The Scotch Black-faced sheep, further afford evidence that where the number of recurrent œstrous periods is increased (beyond the normal number in the home of the breed in question, *e.g.*, the Highlands, in the case of the Black-faced sheep) the duration of the œstrus is shorter,\* while, whether partly or entirely as a consequence of this latter fact, the length of the diœstrous cycle is also less.†

*Superficial Phenomena of the Proœstrum and Œstrus.*

The external signs of the proœstrum in sheep are comparatively slight. The vulva is usually somewhat congested, being slightly swollen, or reddened. There is, too, a slight flow of mucus from the vaginal opening, but only in one instance have I observed any flow of blood. Bleeding of the uterine wall, I find to be extremely slight; but it is in the majority of cases, as I shall subsequently show, present to some degree. External bleeding during proœstrum takes place with many of the domestic animals, and its occasional occurrence in the sheep has been noted by WILTSHIRE (1883) and other authors. The flow of mucus from the vaginal opening, although primarily a characteristic of proœstrum, may continue during œstrus, or even for a very short time during metœstrum.‡

This is due to the extreme shortness of the processes of proœstrum and œstrus in many domestic sheep, the mucus not having had time to escape, although the

\* The longer duration of the œstrus periods of Scotch Black-faced sheep under more extreme conditions (*e.g.*, of climate and altitude) may be taken as evidence that in the perfectly wild state on the high mountain, sheep have a still more prolonged œstrus which does not recur.

† A sheep breeder tells me that in Dorset Horn ewes in the south of England the œstrus *may* recur as often as every eleven days, each œstrus lasting for only about two hours.

‡ That œstrus does not generally commence with bitches until external bleeding is over is a fact well known to dog breeders. But a slight flow of mucus, accompanied by a little blood and a swollen vulva, may continue for some days during œstrus, or even after. Such, at least, was the case with a Dandie Dinmont terrier, in the possession of the present writer, for nearly a week after the performance of artificial insemination. This is owing to the process of proœstrum in the bitch, although longer, being of far greater severity than in the sheep, the resulting discharge consequently requiring a longer period in order to completely escape. The bitch in question was ready to take the dog on the day preceding artificial insemination, and gave birth to pups fifty-nine days afterwards, or sixty days after the commencement of œstrus.

A breeder of Angus and Shorthorn cows (see above, note † on p. 50) informs me that with these animals he has noticed a mucous discharge from the vagina *before* the cows come "on heat" (*i.e.*, before they are ready for service), and that heifers before coming "on heat" for the first time frequently pass blood. Professor EWART tells me that he has noticed a *pro*-œstrous mucous discharge also in the small Shetland ox, which so closely resembles the "Celtic" ox (*Bos longifrons*). These observations are of importance, as they show that the changes characterising proœstrum occur before those belonging properly to œstrus, in cows as in bitches. As pointed out by HEAPE (1900), the prevalent confusion between the two series of changes (*i.e.*, those of proœstrum and œstrus—which are together referred to as the "heat" period) in the lower Mammalia has led to false conclusions regarding the homology between the proœstrum and "menstruation."

internal changes characterising these periods may be over at any rate in most parts of the uterus.

The most obvious superficial phenomena presented by the internal generative organs during proœstrum are the somewhat increased thickness of the uterine wall, and the consequent partial obliteration of the uterine cavity, and the congestion of the Fallopian tubes, which, although often somewhat coloured at other times, may at the "heat" period, throughout almost their entire length, present a deep purple appearance.

The presence of greatly protruding Graafian follicles from one or both of the ovaries cannot properly be regarded as a characteristic of the proœstrum.

The only external indication of œstrus is that afforded by the behaviour of the ewes. At this time they follow the ram, and show a certain restlessness generally. At other times a ewe will refuse service.

#### *Methods.*

Immediately after killing the animal, the body cavity was opened up, and, in the majority of cases, the whole generative tract, including the ovaries, was placed in a 10 per cent. solution of formaline, and kept there until required. The uterus was usually slit open in places, in order to secure a proper penetration. Portions of the uterine wall, after the uterus had been in formaline for at least six days, were cut away, and, after being washed in water for about 12 hours, were hardened in alcohols of gradually increasing strength. After dehydration in absolute alcohol, and clearing in xylol or cedar-wood oil, they were placed in melted paraffin (of a melting point of 58° C.) for periods of time varying according to the size of the specimen. The pieces of the uterine wall were then embedded in paraffin usually of the same melting point, sections were cut by a Cambridge microtome at a thickness varying from 6 $\mu$  to 10 $\mu$ . They were sometimes attached to the slide by Meyer's albumen, but generally I found the sections to become fixed to the slide perfectly well after floating them on to the slide on hot water, and allowing the water to dry off the slide slowly by means of a moderate heat, in the latter case without employing a fixative. They were cleared in turpentine and xylol, stained, dehydrated, and mounted in balsam. The stains employed were hæmatoxylin and iron alum, hæmatoxylin and eosin, methyl blue and eosin, aniline blue, and borax carmine. Of these, hæmatoxylin and eosin were the combination most frequently used.

The sections through the uterine wall were cut transversely to the axes of the uterine cornua. Transverse sections of the Fallopian tubes were also cut and treated similarly.

The ovaries were generally treated in the same way, but sometimes they were cut away separately after opening the body cavity, and fixed in a saturated solution of corrosive sublimate.

*The Histology of the Uterus during the Diœstrous Cycle.*

For general descriptions of the cotyledonary uterus, the reader is referred to the veterinary text-books, to Sir WILLIAM TURNER'S 'Lectures on the Comparative Anatomy of the Placenta' (1876), and to the various papers that have been written on the cotyledonary placenta. For a short account of the transverse section through the uterine wall of the sheep, KAZZANDER'S paper (1890) may be consulted. The latter author shows that the muscle layers can be essentially resolved into the three usual layers, viz., an inner circular layer, a middle longitudinal layer, and an outer layer containing both circularly and longitudinally arranged muscle fibres.

It is also noted by KAZZANDER that the first muscle layer and the connective tissue of the mucosa (*i.e.*, the stroma), are not sharply divided from each other, for some muscle fibres occur in the stroma. These I have noted especially in the intercotyledonary stroma.

The muscle layers do not further concern the subject of this paper, except in regard to the blood-vessels contained in them. These during the proœstrum are liable to some amount of congestion, causing slight distension. I have never seen any evidence of a breaking down of vessels in the muscle layers.

The epithelium bounding the uterine cavity consists usually of a double layer of cubical cells, both on the surface of the cotyledons and of the intercotyledonary mucosa. The epithelium of the glands is frequently composed of more than two rows of cells, especially at their openings, where it may be many layers deep. These openings do not occur on the surface of the cotyledons so much as between them, and at their bases, where they are numerous. No clear line of demarcation could be made out between the cells of the epithelium and those of the stroma tissue.

The stroma or sub-epithelial mucosa is a true connective tissue, and contains numerous blood-vessels, glands in some cases, pigment (situated usually near the surface epithelium), and deeper down in the tissue a few muscle-fibres (as already mentioned), the whole being bound together in a network. The connective tissue framework is, however, very much less obvious in some places (as in some of the smaller cotyledons) than in others. The nuclei are considerably smaller than those of the epithelial cells. I could never satisfactorily make out cell boundaries in the stroma cytoplasm.

The remainder of this section of the paper is devoted to a description of the histological changes through which the uterine stroma passes during a diœstrous cycle, and to a brief comparison of these changes in the sheep with similar changes in other types. The changes in the sheep at any rate relate primarily to the blood-vessels. They may be conveniently described under four heads, as follows :—



- (1.) Period of rest.
- (2.) Period of growth of stroma and increase of vessels.
- (3.) Period of breaking down of vessels and extravasation of the blood in the stroma.
- (4.) Period of recuperation and pigment formation.

Period (1) corresponds to diœstrum, or if the diœstrous cycle in consideration be the first of the sexual season, to anœstrum. Periods (2) and (3) both properly belong to proœstrum, but certain of the characteristics of period (3) not infrequently occur contemporaneously with œstrus. Period (4) corresponds approximately to metœstrum, but recuperation and pigment formation begin actually during œstrus.\* The relation of these periods to diœstrum, proœstrum, œstrus, and metœstrum depends really upon the intensity or abbreviation, as the case may be, of the changes characterising them. These changes appear to differ somewhat in intensity according to the individual, while I have some slight evidence for the assertion that the severity of the process tends to decrease with each successive cycle in the sexual season.

It may be at once noted that the method here adopted of classifying the sheep's uterine stages is similar to that employed by MILNES MARSHALL (1893), and by Mr. WALTER HEAPE (1894, 1897) in grouping the menstrual periods of the human species and of monkeys. The further subdivision in time of these periods in the case of the sheep is scarcely warrantable, owing to the abbreviation of the proœstrous process and the fact that certain characters essential to the occurrence of one period may co-exist with others belonging properly to the next period, present elsewhere in the same uterus.

(1.) *Period of Rest.*—The histological characters of the uterine mucosa at this period, as compared with those of the succeeding periods, may be said to be almost purely negative.

Protoplasmic processes can be seen to be passing from many of the stroma nuclei. But these, though denser in some places than others, show little evidence of division. The slightly denser patches appear arranged quite irregularly.

The blood-vessels are small and comparatively scarce. Those that are present contain numerous red corpuscles, but I have failed to detect leucocytes.

Dark brown pigment, apparently in an amorphous state, lying free in the stroma, for the most part immediately beneath the epithelium, may be present in considerable quantities. According to my experience, this pigment is most frequent rather between and around the bases of the cotyledons than on their surface; but this is by no means invariable, as sometimes the cotyledons themselves, and especially the smaller cotyledons, may appear superficially to be perfectly black with pigment. (*Cf.* BONNET, 1880.)

\* As I shall show later, in the case of the ferret, the œstrus period may extend far into the uterine recuperation stage. (See p. 64.)

I have never observed pigment in the uterus of a yearling sheep, nor in any sheep during the œnestrus period, the above description applying to the uterus during the diœstrus interval. Also I have found pigment to occur more frequently and in greater abundance later in the sexual season (*i.e.*, after several diœstrus cycles have been passed through) than nearer its commencement; but this statement does not apply invariably.

The mode of formation of pigment is discussed under the head of Period (4), where I have referred to the work of BONNET and KAZZANDER.

(2.) *Period of Growth of Stroma and Increase of Vessels.*—The earliest indication of the approach of this period is afforded by a slight multiplication in the number of nuclei in the stroma closest to the epithelium of the cotyledons. But this change is very rapidly succeeded, if not actually accompanied, by an increase, at first scarcely perceptible, in many parts of the uterus, in the size of the blood-vessels in the muscle layers and the part of the stroma in proximity to the muscle layers.

The nuclei in process of multiplication apparently divide into two simply, and without any mitoses, but of this point I could never be certain.

A little later the stroma nuclei become distributed rather more thickly, but more equally, and the vessels not only are larger in size, but increased in number in nearly every part of the stroma. These changes result in the uterine cavity, never large in a non-pregnant sheep, becoming at this period even still smaller.

The glands tend to become somewhat swollen, while a viscous secretion may frequently be detected in their slightly wider lumina.

The uterine epithelium, so far as I have observed, remains completely unaltered in character.

The whole series of changes characterising this period in the case of the sheep are by no means very conspicuous, excepting for the continued increase in the growth of the blood-vessels. This increase is shown in fig. 2 (Plate 7), where the thicker distribution of stroma nuclei in the region of the epithelium of a cotyledon is also represented. Superficially, the above-mentioned changes are not necessarily apparent at all, especially in those cases where the growth is not such as to conspicuously reduce the uterine cavity.

I am unable to form any opinion in regard to whether or not the increase of the blood-vessels is in any sense a result of the growth of the stroma, since the two processes occur almost, if not quite, simultaneously.

(3.) *Period of Breaking Down of Vessels and Extravasation of Blood in the Stroma.*—The congestion is followed (in most cases, at any rate) by the breaking down of some of the blood-vessels, while the growth of the stroma ceases. But the stroma nuclei are still in some places more closely packed than in others, and this thicker distribution continues to occur most frequently in that part of the stroma nearest to the epithelium lining the uterine cavity. Very frequently, the first extravasation takes place from vessels situated immediately below that part of the

stroma where the nuclei are thickest. The vessels in the muscle layers and the adjoining part of the stroma do not rupture at all, so far as I have observed, and the same can be said for the congested vessels of the Fallopian tubes. The rupture in the more superficial portion of the stroma is apparently the direct result of congestion and the consequent strain on the vessel walls.

The blood corpuscles thus set free become scattered in the stroma, where they form irregularly shaped patches and streaks lying a little below the epithelium. Leucocytes are also extravasated with the red corpuscles, but the proportion of the former to the latter, so far as I am able to judge, is not abnormal. I have no evidence of the existence of wandering cells in the uterine stroma apart from what might quite well have been expelled during the rupture of the blood-vessels.

In fig. 3 (Plate 7) is shown part of a section of the mucosa of a cotyledon, with blood-vessels congested, but not ruptured, somewhat deeper in the stroma, and extravasated blood, resulting apparently from very recently broken down vessels situated nearer the epithelium.

Fig. 4 (Plate 7) represents a section showing the nearest approach to a blood lacuna that I have observed in a sheep's uterus during proœstrum. The disappearance of the epithelium at this point is, I think, in part due to faulty manipulation, since it is unusual to find more than about half-a-dozen cells wanting at points where small streams of blood corpuscles were being poured into the uterine cavity. The mere fact, however, of the disappearance of the epithelium opposite a space that was obviously a miniature lacuna, whether it had become denuded naturally or otherwise, is evidence of a weakness at this point in the epithelial wall, due to the pressure exerted from within by the extravasated blood. It is noticeable that in this section, although the stroma presents a fairly normal appearance, capillaries are still wholly absent, while a few extravasated corpuscles are seen scattered in the superficial portion of the tissue.

Formerly I was of opinion that denudation (if only to a slight extent) of epithelium and bleeding into the uterine cavity occurred invariably during the sheep's proœstrum. But material subsequently obtained points to the conclusion that this is not necessarily the case, although it is by no means easy to distinguish between a uterus in which the proœstrum is nearly over and in which there has been little or no bleeding to the exterior, and another uterus in which rupture of vessels has only just begun. But where œstrus has commenced and the uterine epithelium shows every evidence of having remained intact during the proœstrous process, and the blood-vessels deeper in the stroma are not much congested, while blood in an early stage of hæmorrhage is seen in the superficial tissue, it is safe to assume that the severity of the process was not sufficiently great to induce bleeding into the cavity.

According to my observations, the severity of the proœstrous process tends to diminish with each successive diœstrous cycle in the rutting season. Sometimes in a late proœstrum it would appear probable that Period (3) is not reached at all, but

that the congested vessels subside without any rupture. But the matter is complicated by the undoubted fact that in one part of the uterus a more advanced stage may be reached than in another part of the same uterus. Bleeding into the uterine cavity, so far as I have seen, is more frequent through the cotyledons than between them, and is commoner in the case of large cotyledons than with smaller ones.

I have never seen any denudation of stroma during the sheep's proœstrum. The lumina of the glands during this period tend to become still wider, while the glandular secretion may increase in quantity.

(4.) *Period of Recuperation and Pigment Formation.*—The characteristics of this period (as indicated in sections through the mucosa) depend upon the severity of the changes that have occurred in the preceding period, not only in the uterus as a whole, but in the particular part of it under consideration. Where bleeding into the uterine cavity has been considerable, there may be little or no extravasated blood retained in the stroma, and, since bleeding is commoner (at least according to my experience) in the upper parts of the cotyledons than near their bases and between them, one might expect to find less extravasated blood retained in the former parts of the stroma than in the latter. This is by no means invariable, but, as a general rule, I have found it to be the case.

The new epithelium at places where in the preceding period bleeding into the cavity was going on, takes its origin from the adjoining epithelium which did not become denuded. In this way the re-formation of the epithelium in the case of the sheep is easily accounted for, since, as already remarked, only a very inconsiderable number of epithelial cells disappear during the sheep's proœstrum.

Fine branching protoplasmic processes, arising apparently from the nuclei of the stroma, are commonly seen in the early part of the recuperation period, but not where the breaking-down process has been less severe. These processes disappear, to a great extent, at a later stage. The mucosa, as a whole, undergoes a slight shrinkage during the early part of Period (4), but this begins really at the time when bleeding into the cavity occurs. Congestion gradually becomes less and less in the deeper parts of the uterine wall and in the walls of the Fallopian tubes, the vessels being at length reduced to their normal proportions. Meanwhile nuclear division in the stroma is of rare occurrence. Blood-vessels in the superficial part are rarely found in the earlier recuperation stage, and there is no evidence that any of the extravasated blood corpuscles which remain in the stroma are gathered up afresh into the circulatory system, as Mr. HEAPE (1894) states to be the case after menstruation in monkeys. But at a period three days after œstrus it would appear that new capillaries have been formed.

The extravasated blood corpuscles retained in the stroma form pigment, as supposed by BONNET (1882), although at the time of writing the paper referred to BONNET does not appear to have observed extravasated blood in the sheep's uterus. He supposed the extravasation to take place in the deeper part of the tissue during the

“brunst,” the derivatives of the corpuscles being carried in the form of pigment to the more superficial portion of the mucosa by wandering cells. Subsequently he describes these pigment-bearing leucocytes, which he regards as true pigment cells, as breaking down, leaving the pigment free in the mucosa, in the form of light-brown, coarse-grained masses of colouring matter. KAZZANDER (1890) seems to have been the first to have seen the extravasated blood which he correctly describes as occurring mostly in the superficial tissue. But KAZZANDER does not admit the agency of leucocytes, while the appearance of pigment cells he describes as being due to the arrangement of pigment granules around certain of the stroma nuclei. He regards the process of transformation of extravasated blood corpuscles into pigment as occurring free in the tissue and outside the cells.

All gradations from freshly-extravasated blood to completely transformed pigment appear in my sections, but I cannot adopt KAZZANDER'S view that the change takes place independently of wandering cells. On the other hand, the crowding together of little groups of extravasated corpuscles in the early stages often plainly associated with a leucocyte, and the subsequent arrangement of pigment granules in cells having all the appearance of pigment cells, point to the opposite conclusion. At a later period the pigment generally becomes so thickly distributed both immediately below the uterine epithelium and elsewhere, that it is impossible to state definitely whether it is within cells or not, but it has the appearance of lying free in the stroma.

Though the evidence is not quite clear, I find it difficult to resist the conclusion that the transformation from extravasated corpuscles into pigment is worked through the agency of leucocytes which, when laden with pigment, appear as pigment cells. Leucocytes, undoubtedly, are extravasated with the red corpuscles, though not, in the case of the sheep, in any considerable number. In the menstruation of monkeys they are extravasated in very large numbers, and, apparently, quite unnecessarily (HEAPE, 1894). It would appear as if in the latter case the need for leucocytes is obviated by the red corpuscles which remain in the stroma (after denudation), together with the leucocytes being gathered up anew into the circulatory system (HEAPE, 1894). So far as I am aware, there is no record of pigment formation in the uterine mucosa of monkeys.

The amount of pigment formed in the sheep's sexual season depends upon two factors; firstly, upon the extent to which extravasation has occurred in the proœstrous periods,\* and, secondly, upon the quantity of blood retained in the stroma (or, conversely, upon the extent or existence of bleeding into the uterine cavity). Fig. 6 (Plate 7) represents part of a section through the mucosa of a sheep killed towards the end of the sexual season, *i.e.*, a sheep that had passed through several recurrent diœstrous cycles. The animal was killed four days after œstrus. The large quantity of pigment formed was no doubt derived from blood which had been extravasated

\* The quantity of pigment formed must also, of course, depend upon the number of proœstrous periods. This may be inferred from the facts stated below in the same paragraph.

during the several proœstrous periods, and not merely during the most recent of the series. Derivatives of blood corpuscles, associated with wandering cells, by which they were apparently in process of being taken up, and dating from the last proœstrum, were still recognisable. Elsewhere in the same section hæmatoidin crystals were found to occur.

*Literature of the Proœstrum.*—The early papers by BONNET (1880, 1882), and KAZZANDER'S paper (1890) on pigmentation and its mode of formation in the sheep's uterus have been already referred to. Subsequently BONNET (1892), has noted the existence of swelling, congestion, and glandular secretion during the "brunst" of many domestic animals, and made the further statement that in the mare and the sow and ruminants bleeding may take place in the mucosa. ELLENBERGER, in the same volume (1892), makes similar statements, and although not describing extravasation in the mucosa, refers to the pigmentation there in the case of ruminants. (See also BONNET and KOLSTER, 1902.)\*

For an account of the evidence of the existence of the proœstrous period in the Mammalia generally, and a résumé of the literature, the reader is referred to MR. HEAPE'S recent paper (1900), where the question of the homology between the proœstrum and "menstruation" is also dealt with.

MR. HEAPE (1894, 1897) groups the phenomena of menstruation in *Semnopithecus entellus* and *Macacus rhesus* into four periods and eight stages. For purposes of comparison they are set out here:—

A. Period of rest.

Stage I.—The resting stage.

B. Period of growth.

Stage II.—The growth of the stroma.

Stage III.—The increase of vessels.

C. Period of degeneration.

Stage IV.—The breaking down of vessels.

Stage V.—The formation of lacunæ.

Stage VI.—The rupture of lacunæ.

Stage VII.—The formation of the menstrual clot.

D. Period of recuperation.

Stage VIII.—The recuperation stage.

\* Since concluding my paper, I have seen KOLSTER'S memoir in the last issue (Dec. 1902) of the 'Anatomische Hefte,' where pigment cells in the uterine mucosa of the sheep and cow at the "brunst" are described, and BONNET'S original descriptions are to a great extent confirmed. KOLSTER gives a very short account of the histology of the uterus of the sheep, cow, and pig at the "brunst," while BONNET (1902) in the same number briefly describes the uterus of the bitch at this period.

As already remarked, the four divisions into which I have grouped the diœstrous changes through which the sheep's uterus passes are identical with the periods into which Mr. HEAPE has divided the menstrual cycle in monkeys. The chief differences relate to the comparative slightness and corresponding abbreviation of the proœstrous process in the sheep. Thus the growth of the stroma in the sheep is very much less than with the menstrual cycle of monkeys, and occurs simultaneously with the increase of vessels; and in Period (3), although the characters of Stages IV., V., and VI. may all be present, they do not necessarily occur in succession throughout the whole uterus, for while one part of the uterus may pass through Stage VI., in another part bleeding into the uterine cavity may never take place at all. The most important distinction between the two processes lies in the formation of the menstrual clot (Stage VII.) in the Primates. This results from the intensity of the bleeding and the severity of the denudation, which in the case of the sheep's proœstrum scarcely occurs, being, as I have shown above, confined to certain spots on the surface of the mucosa, where a few epithelial cells may be removed. The period of recuperation with *Semnopithecus* and *Macacus*, as described by Mr. HEAPE, differs from that following the proœstrum in the sheep, mainly in the gathering-up into the circulatory system of the extravasated blood corpuscles and leucocytes, and the consequent absence of pigment formation.\*

That the proœstrum of the sheep and the menstrual phenomena of monkeys are physiologically homologous is rendered exceedingly probable in view of the facts cited above, and without reference to other members of the mammalian class. But the probability seems to become converted into a certainty that can hardly be doubted, after making a further comparison with the proœstrous processes of the bitch as described by RETTERER (1892), and of the ferret as observed by the present writer. Special reference must also be made in this connection to Stratz's notes (1898) on the "menstrual" discharges of *Tupaia* and *Tarsius*, in each of which a blood-clot is described, although there appears to be no removal of mucosa tissue beyond the epithelium.

The only account hitherto published of a systematic investigation into the phenomena of the proœstrum in a lower mammal, so far as I am aware, is that of RETTERER (1892), who briefly describes the process in the case of the bitch, as just mentioned.

The uterine mucosa, according to RETTERER, at the beginning of the proœstrum grows to several times its previous thickness. The blood-vessels increase in number and in size, and in the superficial part become so much distended as to cause rupture, which results in a considerable extravasation of blood. Lacuna-like spaces are

\* In his later paper (1900) to which I have referred, HEAPE has extended this scheme of classification to the proœstrous phenomena of the lower Mammalia. In another paper (1898) he has described two menstruating uteri from the human female, showing characters comparable respectively to Stages IV. and VII. of the monkey.

formed, and these discharge the contained blood into the cavity of the uterus. RETTERER refers also to pigment formation from extravasated blood corpuscles in the mucosa. Mr. HEAPE (1900) also states that he has investigated the processes of the bitch's proœstrum to some extent, and that his results confirm those of RETTERER. From these accounts it appears probable that there is no removal of epithelium in the case of the bitch excepting at points where bleeding into the cavity has occurred.

*The Œstrous Cycle in the Ferret.*—I append below, for purposes of comparison, a very short account, which may be considered as of the nature of an advanced abstract of my observations on the œstrous cycle in the ferret.

The ferret is monœstrous, and has one annual sexual season, which, in the absence of the male, consists of a single greatly prolonged œstrous cycle. The proœstrum alone, during which the female will refuse the male, may extend for over three weeks, while the œstrus, or period during which the female will allow copulation, may last for a further period of five or six weeks, and in some cases, if pregnancy be not permitted, seems to extend almost indefinitely. But in a ferret in which œstrus was observed in the beginning of June (at the time when it was procured), the "heat" period was completely over at the end of the first week of July, coition not having been permitted. The sexual season is ordinarily in the spring and first part of the summer. Five bitch ferrets which were obtained in October showed no signs of coming in season until the end of the following March.

Apart from the attitude of the animals towards the male during the œstrus, the only external indications of the existence of "heat" are the very marked swelling of the vulva during both proœstrum and œstrus, and the occurrence of a sanguineo-mucous flow from the genital aperture; the latter characteristic marking especially the close of the proœstrous period, although it may continue to a slight extent after the commencement of œstrus. With two or three individuals I failed to note external bleeding, but it may have taken place without being observed, as it was sometimes impracticable to make regular observations upon the animals during their prolonged œstrous cycle.

The œstrus associated with the swelling of the vulva extends far into the uterine recuperation stage, if not sometimes beyond it; so that similar changes to those which in the sheep characterise metœstrum, in the ferret may occur contemporaneously with œstrus.\*

The histological characters of the proœstrum with the ferret are essentially like those described by RETTERER for the bitch. There is a well-marked growth period, during which the uterine cavity is reduced to about half its normal size, the muccsa being correspondingly increased in thickness. The vessels subsequently become

\* Bears and certain other animals in the Zoological Society's Gardens are said to permit copulation at almost any time; but this is an abnormal condition, and due to the influences of captivity (HEAPE, 1900). Regarding the occurrence of ovulation in the ferret, see p. 70 in the next section of this paper.



extremely dilated with blood. At a later stage large quantities of extravasated blood corpuscles are found scattered in the mucosa, not merely in the superficial part, but deeper in the tissue. But in the ferret also there is no breaking down of vessels in the muscle layers of the uterine wall. The growth and breaking down stages are characterised further by a distinct swelling of the glands. These changes are followed by a denudation stage which seems to be more marked than the corresponding stage in the bitch. In one specimen large parts of the mucosa were found to be stripped of the lining epithelium, and in this case the denudation could hardly be ascribed to faulty manipulation, since numerous groups of detached epithelial cells, in a more or less degenerate condition, and accompanied by a quantity of mucous secretion derived from the glands, were found free in the uterine cavity. In a part of the same uterus there was evidence also of a proœstrous removal of stroma tissue. I find no indication, however, that the loose tissue and blood corpuscles are ever aggregated into a proœstrous clot, and I am inclined to think that the discharge thus composed is usually got rid of very gradually.

In the recuperation period which follows, the stroma is seen to be much reduced, and at the same time to be less compact, while the cavity at the beginning of this period is larger than at any other times during the cycle. This reduction of the stroma tissue is by itself some evidence of removal of its most superficial portion during the denudation period. The blood corpuscles which were retained in the stroma eventually disappear in a manner that might be interpreted as indicating that they are gathered up afresh into blood-vessels, but in the absence of a quite complete series of stages I cannot be certain of this point. But it is evident that they do not become transformed into pigment as with the sheep. A new (or partly new) epithelial layer, composed of flattened cells, is formed during recuperation, and in an early stage of this period I have observed its mode of formation from adjoining epithelial cells in spots where the stroma was still bare. Whether or not at a still earlier stage any portion of the epithelium is renewed from the underlying tissue, as described to be the case for monkeys by Mr. HEAPE (1894, 1897), I am unable to say positively, but the relation between certain tracts of epithelium and the adjoining stroma suggests the correctness of this view in the case of the ferret.

It is clear from the facts summarised in the preceding paragraphs that all the stages into which Mr. HEAPE first divided the menstrual cycle of *Semnopithecus* are represented in the œstrous cycle of the bitch (RETTGER, 1892), and in that of the ferret, with the exception of Stage VII. (the formation of the menstrual clot), the characters of which in the case of the latter animals are only partially recognisable. It is apparent also that the proœstrous process in the carnivore, in regard to its severity, is approximately intermediate between that of the sheep, as described above, and the menstruation of monkeys, as worked out fully by Mr. HEAPE. But the œstrous cycle of the ferret differs both from the diœstrous cycle of the sheep and from the menstrual cycle in the Primates in its far more extended duration.

*Ovulation, with Notes on the Atretic Follicle, and the Causes of Barrenness.*

Ovulation in the sheep, as in the majority of the lower Mammalia, so far as is known, takes place during the sexual season. In the early summer months (the anæstrous period with the breeds studied) the ovaries are more compact than later in the year, and show little or no indication of maturing follicles or corpora lutea. With the approach of autumn, the Graafian follicles are seen in varying degrees of protrusion from the surface of the ovaries, while in the sexual season ripe (or nearly ripe) and recently ruptured follicles can be found in the ovaries of nearly every non-pregnant ewe.

The ovaries of lambs born in the previous spring, as autumn approaches present similar superficial changes, the sexual season with them beginning about the same time as that of older sheep.

In sections through ovaries of sheep killed during the anæstrous period it is also unusual to find follicles in early stages of degeneration. Those that do occur, so far as I have observed, are follicles that have only reached a comparatively early stage of growth, and not such as have matured and failed to rupture.

When ovulation has recently taken place, it can usually be readily detected superficially by the presence of a blood-clot on the surface of the ovary, or the existence of a pit-like depression indicating the point of rupture of the follicle, which remains open for at least four days. But to avoid all possible confusion, of which in other animals there has been much, between true corpora lutea in their early stages of development and atretic follicles which have never ruptured, I considered it advisable to undertake further the study of the changes undergone by the discharged follicle in the sheep. These changes are described under the heading, "The Formation of the Corpus luteum" (p. 78).

My observations regarding the stimulus necessary to induce ovulation may be conveniently described in the form of a series of experiments. As already mentioned, the periods of œstrus were detected by the attitude of the ewes towards the ram, which, when required for the purpose of the experiment, was rendered temporarily incapable of insemination.

*Experiment 1.*—A virgin ewe (a cross between a Cheviot ram and a Border Leicester ewe) was killed a day after that its first œstrus had been observed, coition not having been permitted. It was found that ovulation had recently taken place. The subsequent examination of the ovaries pointed to the fact that there had been no previous ovulation. The histology of the discharged follicle is described in the following section of this paper as a twenty-four hour stage in the development of the corpus luteum.

*Experiment 2.*—A Scotch Black-faced sheep early in the breeding season was noted to be in a state of œstrus, but coition was not permitted. Two days later the ewe was killed, when it became evident that a follicle had recently ruptured.

There was no evidence that ovulation had occurred previously during that sexual season, neither had a previous œstrus been recorded that year. The sheep was a four-year-old, and had given birth to a lamb the previous year. This experiment, then, was a repetition of Experiment 1, but with a sheep that was not a virgin, the result in regard to the occurrence of ovulation being the same in each instance. The discharged follicle is described below as a fifty-hour stage in the formation of the corpus luteum.

*Experiment 3.*—œstrus was noted in a Scotch Black-faced sheep shortly after the beginning of the sexual season. After an interval of fifteen days, œstrus was again noted. The sheep was killed four days later, when a newly discharged follicle was found in one of the ovaries. By a comparison with other stages in the formation of the corpus luteum, it was stated that the follicle in question had ruptured about the time of the second œstrus observed. Coition had not been permitted during either œstrus.

*Experiment 4.*—œstrus was noted in a Black-faced ewe, and was observed to recur after fifteen days, and to recur again after a further interval of sixteen days. The sheep was killed about seven hours after that the third recurrent period of œstrus had been noted, and without being served. Ovulation had very recently taken place. The newly discharged follicle is described below (p. 79) as a seven-hour stage in the formation of the corpus luteum. I found subsequently in the same ovary what I believe to be the remains of a follicle that had discharged during one of the previous œstrous periods.

This and the preceding experiment show that sheep can ovulate independently of coition at other œstrous periods than the first of the sexual season. In neither experiment were the sheep virgin animals.

*Experiment 5.*—œstrus was noted in a Black-faced ewe (not a virgin) in the beginning of February, and the ewe was served by a ram of its own breed. Sixteen hours after coition the ewe was killed, and a very recently discharged follicle was discovered. Subsequently, on cutting one of the ovaries into sections, I found (in addition to the young corpus luteum which is described in the next section) a degenerate follicle, which, judging from its size and position, which were those of a nearly ripe follicle, and its state of degeneration had probably attained complete ripeness before undergoing atresia. The membrana granulosa had almost entirely disappeared, its few remaining cells being in an advanced stage of degeneration. Very little ingrowth of connective tissue from the thecæ had occurred, and what had was looser and more irregular than the normal ingrowth. The cavity contained a fluid resembling the liquor of the Graafian follicle, but staining somewhat more deeply. There was no blood-clot, such as sometimes occurs in atretic follicles in the rabbit's ovaries, nor any indication that there had been a breaking down of vessels, or rupture to the exterior. No recognisable trace of the ovum was found, but this may have been due to the fact that some sections were unfortunately lost in the cutting.

The special interest attaching to this observation lies in the possibility of the atretic follicle having attained complete maturation and afterwards undergone degeneration owing to want of sufficient stimulus to induce ovulation.

*Experiment 6.*—Œstrus was noted in a Scotch Black-faced ewe (not a virgin) in the beginning of February, but coition was not permitted to occur. On the following day the ewe, which was no longer in season, was killed. There was a considerably protruding follicle in one ovary, but no indication of a recently ruptured follicle in either ovary. On the other hand, it was quite clear that ovulation had not occurred during the observed œstrus. The animal had not been under strict observation before this œstrus, so it is unknown how many diœstrous cycles had been passed through.

In three other experiments in which the sheep were killed within seven hours of coition, ovulation had not yet taken place, while in a further case also in which the sheep was killed about twelve hours after the detection of œstrus, ovulation had not at that time occurred. But in the latter experiment coition was not permitted. In no cases, other than those mentioned above, after that a sheep in which œstrus had been noted shortly before was killed, did I fail to find one or more recently discharged follicles.\*

It may be concluded from the above-described experiments that hill sheep, or half-bred hill sheep, whether virgins or otherwise, under ordinary conditions, ovulate in response to a stimulus during œstrus (or possibly occasionally during the proœstrum), and that they can do so independently of coition during any œstrus of the regular sexual season; but that ewes in which, for one reason or another, œstrus occurs at a considerable time after the termination of the regular sexual season, may require the additional stimulus imparted by sexual intercourse in order to bring about ovulation. There is also evidence that where coition occurs at the beginning of an œstrus period, it may provide a stimulus inducing ovulation to take place a few hours earlier than it might in the absence of coition; or, in other words, that if ovulation has not already occurred during an œstrus, the stimulus derived from the act of coition may, so to speak, hasten the ovulation.

The statement concerning the non-occurrence of spontaneous ovulation in sheep during œstrus at other times than the normal sexual season requires further explanation.

On the supposition that the sheep mentioned in Experiment 6 had come "in use" at the usual time for Black-faced sheep, kept under favourable conditions, and that

\* The following statement occurs in the 'Preliminary Communication,' p. 137 (1901): "In no case after a sheep in which œstrus had been observed was killed in order to obtain a stage in the development of the corpus luteum was the corpus luteum not found." At the time of writing this passage I had obtained only one very early stage (a "seven-hour stage") in the formation of the corpus luteum. Subsequently I have had considerable difficulty in getting very early stages, owing to their being no fixed time for ovulation during œstrus.

each of the diestrous cycles lasted sixteen days, the observed œstrus was the seventh of the sexual season. This would imply a more extended series of diestrous cycles than is usual among ewes of this breed kept under any conditions. But it may be that while some ewes have such an increased breeding capacity, yet the stimulating power at their disposal during an exceptionally late œstrus is so reduced that without coition and the presence of spermatozoa in the uterus it is insufficient to cause ovulation. On the other hand, the ewe may not have come "in use" first until very much later than the usual season, in which case the need for a special stimulus was associated with an individual lack of breeding capacity. The only other alternative accounting for the late œstrus, and one which from other evidence appears to me the most probable, is that the sheep became pregnant to a ram at an early œstrus, and soon after aborted, subsequently coming in season again. This explanation, also, implies a want of generative vigour, whether temporary or otherwise, on the part of the ewe in question.

Either of these explanations will account similarly for the presence of the atretic follicle described in Experiment 5. This experiment also serves to show that with the additional stimulus set up by sexual intercourse, ovulation can occur during an œstrus outside the normal sexual season.

Whether or not the relation between copulation and ovulation is the same for all breeds, I have no means of knowing. But seeing that it can vary with circumstances with one breed, it is possible that different breeds show different degrees of adjustment in this respect. However this may be, the fact of the occurrence of spontaneous ovulation during œstrus in sheep, belonging to some breeds at least, is of importance, since it indicates the possibility of obtaining successful results by artificial insemination.

According to HAUSMANN'S description (1840) it would appear that sheep ovulate normally as a result of coition, but this conclusion may be due to the fact that HAUSMANN'S observations were upon sheep which had been served before ovulating; and, as I have shown above, copulation not infrequently does occur prior to ovulation.

*Ovulation in other Mammals.*—The rabbit, it would appear, is the only other animal which is known to vary in regard to the stimulus necessary for ovulation. Mr. HEAPE'S experiments (1897) show that in the virgin rabbit both copulation and the presence of spermatozoa in the uterus may be essential, while the number of atretic follicles to be observed in almost any rabbit's ovaries renders it probable that it is not only virgins which fail to ovulate spontaneously when "on heat." FLEMMING (1885) states that he found at least twenty degenerating follicles in each of the ovaries of two virgin rabbits, and also records atretic follicles in an animal that had given birth to young, and in two old animals. On the other hand, WEIL (1873) makes the statement that ovulation can occur without coition in doe rabbits shortly after parturition, and OTT (1882), and, more recently, GRUSDEW (1896) have obtained successful results

from the artificial insemination of rabbits, but not in a high percentage of cases. Professor EWART (1901) has described an experiment with a grey doe rabbit (a virgin) which was served (although not in season) forty days before parturition, or ten days before ovulation, thirty days being the usual period of gestation with the rabbit. This case seems to show that sometimes, even with virgin does, the presence of spermatozoa in the rabbit is enough to induce ovulation, for the ovulation can scarcely have occurred as a result of a stimulus imparted by coition, since the rabbit was served ten days previously, and before it had come in season.\*

Most other mammals in which the subject has been investigated are said to ovulate spontaneously during œstrus. SOBOTTA (1895) has shown that this is so with mice, while it follows that those animals which can become pregnant from artificial insemination during œstrus, also can ovulate without coition. Such is the case with mares and donkeys and cows (HEAPE, 1897, 1898),† with which artificial insemination is now fairly widely practised. Artificial insemination has also been performed with successful results on bitches (MILLAIS, 1895 ; HEAPE, 1897, &c. ; see also note on p. 54), and also on the human female (HEAPE, 1897, &c.), but not, so far as I am aware, upon other Mammalia.

From my observations on ferrets, I have found that ovulation does not take place without coition, and, unless the female ferret is warded at the right time for ovulation to occur, the follicles and contained ova undergo atresia. But, notwithstanding the ovulation being abortive, the œstrus persists until long after the time for ovulation has gone by, so that a ferret, when warded too late in the season, fails to become pregnant. This may be the explanation of the fact noted by ROBINSON (1893) that coition very frequently does not result in pregnancy, although the ferret may have copulated more than once during œstrus. Thus the persistence of the œstrus which continues far into the recuperation stage of the uterus, or beyond it, is associated with numerous degenerating follicles in the ovary, which, in the natural condition, would no doubt have discharged at an earlier period, under the influence of the increased stimulus of coition. A female ferret which I inseminated artificially during œstrus did not become pregnant, owing, probably, to the presence of the sperms in the uterus without the act of coition failing to induce ovulation.

In many bats ovulation is described as occurring at quite a different time of the year from œstrus, copulation generally taking place in autumn and ovulation in spring (VAN BENEDEN and JULIN, 1880), there being sometimes as long as six months between them. The sperms are said to retain their vitality until the spring ovulation.

\* The doe here referred to was a quarter-bred wild rabbit, and in colour and disposition resembled its wild grandparent. It has occurred to me that this fact possibly may explain the occurrence of ovulation in a virgin doe without the additional stimulus of coition. A wild rabbit may conceivably have greater stimulating power at its disposal than one might expect to find evidence of in breeds that have long been domesticated.

† Donkeys are not referred to by Mr. HEAPE in this connection, but artificial insemination has been performed on donkeys to my knowledge, and has resulted in pregnancy.

It follows that in bats ovulation can occur without even the stimulus of œstrus, unless the existence of the spermatozoa in the uterus may in some way provide a stimulating influence. But ovulation could only occur if there were ripe follicles present in the ovary, and the ovary in the winter months (during hibernation) is said to be in a state of quiescence (VAN DER STRICHT, 1901). Lately, however, SALVI (1901), from observations upon the bats in the Grotta dell'Inferno, near Sassari, says that they copulate in spring as well as in autumn, the autumn sexual season having long been known.

According to STRATZ (1898) the ova of *Tupaia* become mature at "menstruation." In *Sorex* also the mature ova are produced during the "heat" periods, while at other times the follicles undergo atresia (STRATZ, 1898).

The periods for the occurrence of ovulation in the Primates have been the subject of a considerable amount of controversy, the question being discussed at some length in three papers by Mr. WALTER HEAPE (1894, 1897, 1898), who shows that menstruation and ovulation are to a very large extent independent functions. Mr. HEAPE points out further that whereas, in both monkeys and the human species, menstruation may occur periodically all the year round, in monkeys there is a limited time for conception and ovulation, while in civilised woman this period is not limited to any particular time of the year, although there is evidence that primitively man agreed with the lower Primates in having a definite sexual season.\* The fact is also noted that conception (and, consequently, ovulation) can occur in the human female, even although there may never have been a menstruation. The discussion of these facts is inseparable from the question regarding the homology between menstruation in the Primates and the proœstrum in the lower Mammalia, a subject to which reference has been made above.

*The Atretic Follicle.*—The sheep referred to in Experiment 6 furnished the only unequivocal case of a follicle failing to rupture during an observed œstrus,† although

\* HEAPE, in a later paper (1900), gives a very brief *résumé* of the evidence (derived largely from the writings of PLOSS and WESTERMARCK bearing on this subject. Very recently Messrs. ANNANDALE and ROBINSON have kindly furnished me in advance with the following statement, which has not yet appeared :—

"One of the women of a Semang or aboriginal tribe in the Siamese Malay State of Jalor volunteered the information that the women of her race only had children about March, that is, immediately after the wet season, and that each woman normally had a child every year at this season, as long as she was capable of child-bearing."

I am indebted to Messrs. ANNANDALE and ROBINSON for permission to make use of this very interesting information, which, so far as I am aware, is among the most direct evidence of a restricted sexual season in a primitive race. (Cf. "Fasciculi Malayenses, Anthropology," Part I, now issued.—May 15th.)

† It is possible that the follicle referred to was not sufficiently advanced towards maturation to admit of rupture, since it did not show any indication of atresia, so far as I observed. But this may quite well be explained by the fact that the ewe was killed only on the day following the detection of œstrus, and possibly but a few hours after the termination of that œstrus; so that the follicle might not have begun to degenerate. I am unable to state when the polar bodies are extruded in the case of the sheep.

the histological appearance of the atretic follicle described in the preceding experiment made it probable that it had undergone degeneration owing to insufficiency of stimulus when in a condition for ovulation. Numerous cases, however, of follicles becoming atretic before, and usually a considerable time before, attaining their maximum development have come under my observation.

With the sheep, the follicle undergoing atresia can be identified by the following characters, which distinguish it from the corpus luteum or discharged follicle :—

(1.) There is no indication of any rupture to the exterior.

(2.) The ovum, being retained in the follicle, degenerates, losing its regularly circular shape as seen in section, becoming shrunken, and eventually disappearing altogether.

(3.) The follicular epithelium, instead of hypertrophying, as in the formation of the corpus luteum, degenerates, the chromatic substance at one stage appearing in the form of fine points in the cytoplasm, much smaller than nuclei. The cells, in various stages of degeneration, become scattered in the liquor, and often closely resemble polynuclear leucocytes. I have never noted division among the epithelial cells, nor deposition of fatty particles. Later, in atresia, the remains of the epithelial cells become unrecognisable, finally disappearing altogether.

(4.) The connective tissue wall does not proliferate to form a network among the epithelial cells. Generally there is no growth inwards from the theca until the epithelial cells are in a very advanced state of degeneration or have entirely disappeared. Eventually there is a loose ingrowth of connective tissue to fill the cavity.

(5.) There may be a slight breaking down of blood-vessels early in atresia, so that blood corpuscles are scattered in the follicular cavity. (I have never seen a blood-clot in a sheep's atretic follicle, and only noted a few scattered corpuscles in a single instance.)

Where atresia occurs in very young follicles, they can obviously be distinguished from developing corpora lutea by their small size.

I have made no attempt to obtain a complete series of stages illustrating the degenerative changes which the atretic follicle passes through in the case of the sheep. These changes have been described for various Mammalia (chiefly rabbits, cavies, and other rodents) by SCHULIN (1881), FLEMMING (1885), SCHOTTLÄNDER (1891, 1893), HENNEGUY (1894), JANOSIK (1896), and (for bats), VAN DER STRICHT (1901), besides some other writers. My observations, so far as they go, are in general agreement with their far more detailed accounts, which, in the main, are in accord with one another. It would seem probable, however, that HENNEGUY (1894) has mistaken for the membrana granulosa the thickened theca interna, since the ovum described is obviously far advanced in atresia. SCHULIN (1881) and also JANOSIK (1896) appear to regard the follicular epithelial cells as being actually converted into leucocytes, which, as noted above, they frequently closely resemble when undergoing



atresia. FLEMMING (1885), on the other hand, denies the existence of leucocytes, pointing out that none occur in the theca, and SCHOTTLÄNDER (1891) clearly distinguishes degenerating epithelial cells from leucocytes. SCHOTTLÄNDER (1891) also describes atresia as occurring by fatty degeneration as well as by chromotolysis.

The presence of a degenerate ovum cannot apparently, by itself, be regarded as an absolute indication of an atretic follicle, since SOBOTTA (1896, 1897) has recorded instances of follicular discharge in the mouse and the rabbit, in which the ova were accidentally retained in the cavity, the follicles nevertheless forming true corpora lutea; while VAN DER STRICHT (1901) describes a similar case of retention of the ovum in *Vesperugo*, where part of a ruptured follicle possessed the characters of a young corpus luteum and another part those of an atretic follicle.

Atresia is commonly stated to be most frequent during pregnancy, but it occurs at other times also (compare JANOSIK, 1896, and others\*), and according to my observations on the sheep, it is commonest among follicles that have attained to dimensions from about one-eighth to one-half of those of the mature follicle. In a complete series of sections through the ovary of a sheep killed in the month of August (during the œstrous period) I failed to discover a single atretic follicle, nor did I find any in the ovary of a yearling lamb killed at about the same time. In another sheep's ovary during anœstrum I have noted atresia. But my inability to find young degenerating follicles oftener was probably in part due to my observations in ovarian histology being mainly confined to material obtained during the sexual season or very early in pregnancy. But at these times to find young follicles undergoing atresia is no very rare occurrence, for I have noted three or four in a single ovary, or even within the limits of a single section.

It is difficult to avoid associating the degeneration of developing Graafian follicles in healthy ewes with detrimental environmental influences, and more especially with scarcity of food supply, or of the kind of food supply necessary for breeding stock. When occurring to any considerable extent, it must inevitably reduce the number of ripe follicles in the sexual season, and thereby raise the barrenness percentage.

The question, then, concerning the causes producing follicular degeneration is not without its practical side. To this subject I shall return presently.

*Number of Follicles Discharging at an Œstrous Period.*—I give below details of observations in regard to the number of follicles which discharge at a time (*i.e.*, during a single œstrus), the figures being derived from the examination of the ovaries of fifty-five Scotch Black-faced ewes, or half-bred Black-faced ewes, in which ovulation had occurred. The results were obtained either by counting the follicles shortly after rupture (when the number which have recently discharged is usually perfectly obvious), or by noting the number of corpora lutea in pregnant animals. All doubtful cases (such as when it was impossible by superficial examination to make

\* SCHULIN (1881) says that atresia may set in at any stage, and even in the primordial follicle.

out clearly how many corpora lutea were present) were passed over and not included.\*

Number of sheep in which a single follicle had ruptured at ovulation . . . . .	42
Number of sheep in which a single follicle in each ovary ( <i>i.e.</i> , two follicles) had ruptured at ovulation . . . . .	7
Number of sheep in which two follicles had discharged in one ovary, but none in the other, at ovulation . . . . .	5
Number of sheep in which two follicles in one ovary and one in the other ( <i>i.e.</i> , three follicles) had discharged at ovulation . . . . .	1
	55
Total . . . . .	55

Thus in less than 24 per cent. of the cases examined where ovulation had occurred, was more than one follicle found to have discharged.†

The number for which the percentage is calculated is probably too small to support any general conclusions, but taken in conjunction with other considerations, is not altogether unsuggestive.

Mr. HEAPE'S records (1899) for 77,850 ewes belonging to 275 flocks of various breeds, obtained for the year 1896—97, show an average return of 30·14 twin lambs per 100 ewes. No similar records, so far as I am aware, have ever been made for Scotch or other mountain sheep; but there can be no doubt that with these the percentage of twins is a good deal less than among Lowland or Down breeds. A sheep farmer informs me from his own experience that from among fifty score of Black-faced sheep not more than forty pairs of twins are to be expected, while among Cheviots there may be 25 per cent. twins, and among half-bred Black-faced about the same number, but the percentage may be higher. My informant states further, in reference to both Cheviots and Black-faced sheep, that the twin percentage varies considerably according to whether the ewes are kept on lowland pasture or on the side of the hill, in the latter case there being practically no twins produced. Statements made to me by other sheep-breeders are in general agreement with these rough estimates, but the twin percentage for pure Black-faced sheep is sometimes said to be higher, the number of twins reaching 10 or 12 per cent.

It is of course obvious that where only one follicle ruptures at ovulation, only a single lamb can be produced, and in view of the percentage of follicles discharged (as

\* The majority of these were from slaughter-house material obtained indiscriminately, but I have also counted in discharged follicles (described elsewhere in this paper) from sheep that had been under observation.

† In the case of some of the slaughter-house material, I was unable to determine whether the sheep were pure Scotch Black-faced, or crosses in which the character of the Black-faced breed was dominant. But with pure Black-faced sheep alone, there is evidence that the percentage in which two follicles discharge is less, seeing that with the seven ewes (included) that had been under observation, only one follicle had discharged in each instance.

noted) being scarcely, if at all, in excess of the usual percentage of lambs produced\* for the breeds in question, the converse of this statement is most probably also generally true. And since scarcity of twins is intimately associated with barrenness, as shown by Mr. HEAPE (1899), and that habitually (*i.e.*, constitutionally) barren ewes† are a rarity, it is rendered exceedingly probable that infertility generally in sheep results from the absence (or great scarcity) of ripe follicles; that is to say, that in dealing with the causes of barrenness for the purposes of practical sheep breeding, only a single question need be considered, *viz.*, the scarcity of Graafian follicles available for ovulation at "tupping time" (*i.e.*, during the sexual season).

It is a common practice with some sheep-farmers to give the ewes an extra supply of food (such as turnips, cake, or corn, in addition to the pasture they ordinarily live on) shortly before the "tupping season," so as to have them in good condition by the time they are ready to take the ram, the object being to increase the percentage of births in the succeeding lambing season. There is, however, some difference of opinion as to the advantages gained by thus "flushing" the ewes, especially in those cases where, after "tupping time," they are placed on mere sustenance diet. SCOTT, in an article on "The Practice of Sheep Farming," while advising breeders to have the ewes in good thriving condition before tupping time, says that this "should not be accomplished by a process of hurried and temporary stimulation." Although deprecating "flushing," he admits that it may increase the number of births at the ensuing lambing season (but "the extra number of lambs raised in any one year by this system is on the average not very great"), while "from actual test" he is convinced "that ewes which have been flushed one year are never so prolific the next."

When it is remembered that the ripening of the Graafian follicles is a process which goes on not merely during "tupping time" and for a short period before but throughout the whole of the animal's lifetime, it becomes obvious that the practice of "flushing" must lead in different individuals to different results, depending upon age, and the previous conditions to which the ewe has been subjected in regard to food supply and environment, and so upon the extent to which follicles have degenerated, the degrees of ripeness of the developing follicles, and consequently the number which can be brought to maturity in the approaching sexual season.

It follows also that "flushing" does not necessarily have the desired result even in the following lambing season. That this is so is shown by two experiments carried out for a very different object‡ under the direction of GIROU, by whom they are described (1827).

\* Allowance must be made for the possibility of one embryo aborting in a small proportion of cases.

† No case of a constitutionally barren sheep has come under my notice. HEAPE (1899) says that "it is very generally conceded that 2 per cent. is a liberal allowance for constitutionally barren ewes, and that anything over 2 per cent. experienced is due to other causes."

‡ GIROU's experiments were to determine the causes influencing sex. Consequently in stating the

In the first experiment, two flocks during the tugging season were placed respectively under different conditions, the one being supplied with more abundant nutrition than the other. The following is the result of the subsequent lambing, the ages of the dams being also given :—

Flocks under more favourable conditions.		Flock under less favourable conditions.	
Age of dams.	Number of lambs.	Age of dams.	Number of lambs.
2 years	40	2 years	10
3 "	45	3 "	29
4 "	26	4 "	47
5 " and over	26	5 " and over	49
Total . . . 137		Total . . . 135	

The number of sheep in each flock does not appear to be stated, but they are described as having been of approximately equal size. Each flock was provided with two rams. In the flock given more abundant pasture there were three cases of twins, but none in the other. No cases of barrenness or abortion are mentioned (this not concerning the subject of GIROU'S investigation); but since the flocks are described as being composed about equally of ewes of all ages, it may perhaps be inferred that some of the younger sheep among those given less abundant food were barren. The total number of births in each flock is shown to be very nearly equal.

In another experiment described by GIROU (1827) a flock of eighty-four ewes was divided into equal parts, one-half being placed on much richer pasture than the other during the sexual season. The result in regard to the number of lambs produced was exactly the same in both instances, there being forty lambs born in each half of the flock. Where the pasture was the richer there were two cases of twins, with the other half one case of twins.

It is natural to assume that the fertility of young sheep is more sensibly affected by an increased food supply and more favourable environment in the sexual season than is likely to be the case with the older ewes, since with the former there has been less time and opportunity than with the latter for the operation of previous environmental influences, which, if adverse, would have retarded the ripening or brought about the degeneration of such of the Graafian follicles as might otherwise have become mature. I have pointed out that the first of GIROU'S experiments affords some evidence of the truth of this deduction, while the opinion which Mr. HEAPE (1899) says has not infrequently been expressed to him "that shearing ewes are more liable to barrenness [presumably in unfavourable seasons] than older

number of lambs produced, GIROU gives the number of male and female lambs respectively. As the sexes of the lambs do not concern the subject of the present paper, I have added the numbers of males and females together, and given only the totals.

ewes," points in the same direction, although the returns submitted to him do not show any proof of this except for the Dorset breed. For these "there is some evidence that in unfavourable tugging seasons, during great heat and scarcity of green food, the young ewes are more likely to be affected than the older ewes, and less likely to be in a satisfactory breeding condition."

Of more doubtful bearing on this subject is SCOTT's statement, already quoted, that ewes from which an extra number of lambs have been raised by flushing one year are less prolific at the subsequent lambing, or the fact referred to in the section of this paper on the "Œstrous Cycle," that those Limestone sheep which produce lambs twice during one year are barren in the year following. Such facts as these are usually ascribed to general strain on the reproductive system, but when this strain is said to cause subsequent barrenness (as distinct from abortion or "slipping lamb"), I would suggest that the barrenness is due to a premature but partial ripening of the Graafian follicles succeeded by an unusual amount of follicular atresia.\*

The general practical conclusion to be drawn from the above considerations is that it is better to keep the ewes in fair thriving condition, as continuously as possible, than to submit them to an unnatural process of stimulation by "flushing" shortly before and during the sexual season, while maintaining them on mere sustenance diet at other times of the year.

There is another point of some practical importance which deserves notice. It is sometimes asserted by breeders that the fertility of sheep is affected by the ram employed (*e.g.*, that the production of twins or triplets in place of single lambs is in some way due to the use of particular rams). In the light of the facts stated above regarding the production of ova, and remembering the far greater reproductive capacity of the ram as compared with the ewe, these assertions (excepting where they can be shown to apply to the causes of abortion) may be disregarded; and as a practical matter, the question of fertility (at least ordinarily) may be considered as one affecting the ewe alone. At the same time, it is obvious that to ensure copulation occurring with every ewe, the proportion of ewes to rams must not be too high, while there is evidence that the sexual activity of the ram is liable to vary, both individually and according to the breed.

The Border Leicester and Scotch Black-faced rams afford an example of this variation. It is a common practice in Scotland with breeders of crosses between these two varieties of sheep to keep Black-faced rams to serve those Black-faced ewes which in the same sexual season have failed to become pregnant to a Border Leicester ram,

\* I have never met with a case of œstrus occurring with a ewe in the absence of protruding or very recently discharged follicles. Whether or not œstrus does ever occur with sheep without the possibility of ovulation (*i.e.*, with barren sheep) I am unable to say. But to assume that it does not ordinarily do so is not to assert that œstrus depends upon ovulation, since the two phenomena may both be results of the same or similar causes. In some Mammalia, as has been pointed out by HEAPE, ovulation and œstrus can occur quite independently.

the failure being merely the result of the latter not copulating, and not due to infertility on the part of the ewes. I am informed that with such cases, in the lambing season, as many as half the lambs produced may be pure Black-faced, and from ewes which would have failed to bear, had the work been left entirely to Leicester rams.\*

The statement that Suffolk and Wensleydale ewes are more prolific with rams of their breeds than with other rams (HEAPE, 1899) may perhaps be similarly explained.

On the other hand, with Dorset Horns, Mr. HEAPE describes the ewes as being less barren with Hampshire Down rams than with Dorset Horn rams. Referring to a general instability of the reproductive system of Dorset Horn ewes, and their need for some special stimulus, he appears to suggest that this is supplied by the use of a Down ram. I think that it is more probable that what in these cases is described as barrenness is in reality very early abortion, an unavoidable element of error, which Mr. HEAPE recognises as present in his statistics for sheep generally, (though he states that he believes the error is, as a rule, small) being in the case of the Dorset sheep somewhat larger than elsewhere in the returns given. That this is the true explanation is rendered the more probable by the facts also recorded by Mr. HEAPE, "that, of all breeds, the Dorset Horns appear to be most liable to abortion," and further, that in some cases, "Dorset Horn ewes which slip lambs got by Dorset Horn rams will bear lambs got by Down rams." I would suggest that in this case the cause of abortion lies, not so much with the ewe, as in want of vitality in the embryo, which when aborted early is absorbed in the uterus, escaping the notice of the shepherd; and that where the Dorset Horn sheep have been served by Down rams, there is less abortion as a result of increased vigour on the part of the cross-bred young.

#### *The Formation of the Corpus Luteum.*

The ages of the corpora lutea in this investigation were reckoned from coition, or where coition did not or was not known to have taken place from the time when œstrus was observed, the sheep being killed at various intervals of time afterwards. The facts recorded in the previous section of this paper indicate that ovulation only takes place during the œstrous periods (or very possibly in some instances in the proœstrous periods which immediately precede them), while the observed relation, as described below, between the condition of development of the corpus luteum and the interval that had elapsed between the œstrus noted and the killing of the sheep in question, is by itself strong evidence that the two phenomena occur together. But the method adopted of reckoning the ages of the developing corpora lutea is inevitably a somewhat arbitrary one, since, as already

\* The failure of the Leicester rams here must be partly due to their being less suited to the Scotch climate in the latter part of the sexual season of Black-faced ewes.

remarked, there is no definite time during œstrus at which ovulation occurs, while an œstrous period may extend over several hours. Consequently two recently discharged follicles, although of the same age, according to the method of reckoning, may present slightly different degrees of development\*.

I proceed to describe the stages which I have obtained in the formation of the sheep's corpus luteum. When coition was known to have taken place, the fact is recorded.

The corpus luteum obtained from an animal killed *seven hours* after coition differs from the unburst follicle in its size and in the fact that the ovum and discus proligerus have been discharged. It is a little more than half as large as the mature follicle, and consequently does not protrude from the surface of the ovary. On the other hand, the point of rupture is situated somewhat in a depression. Very little blood is found remaining in the cavity of the follicle, but a few scattered corpuscles are seen in the membrana granulosa, which was not discharged with the ovum and discus proligerus. The blood corpuscles are apparently derived from vessels, the walls of which have broken down, not merely near the point of rupture of the follicle, but to a less extent around the whole theca interna. The greater quantity of the blood corpuscles set free from the vessels at the rupture of the follicle had been discharged to the exterior, forming a stain, in which the blood had not yet clotted, on the surface of the ovary in the region of the depression communicating with the follicle's cavity. This latter contains a fluid resembling in all respects the liquor folliculi. The membrana granulosa at this stage of development is approximately twice the average thickness of that of the undischarged ripe follicle, some of the cells having increased in size, while others, especially those nearest to the periphery, retain the characters of the original follicular epithelial cells. Evidence of recent mitosis among these cells is not uncommon, though not so frequent as with the epithelial cells of the Graafian follicle. In this specimen there is no sign of any growth inward of connective tissue from the theca interna, which, except at certain points where blood-vessels had ruptured, did not so far appear to present any change.

In another seven-hour stage, the development of the corpus luteum is in a slightly more advanced state. Ingrowths, at this period resembling little bud-like projections arising from the theca interna, are seen on the inner side of that layer, and although blood-vessels are also beginning to grow inwards with the connective tissue, the proliferation of the theca cell is not as yet very great. The anastomosis of fusiform cells, which arises from division of the cells of the theca, and which eventually comes to surround nearly every one of the follicular epithelial cells, at this stage has not begun. The membrana granulosa already shows considerable hypertrophy, but still retains the irregularity of arrangement and want of cohesion which results from certain of its component cells having shifted their position at the time of

\* See p. 68.

rupture. This irregularity is more marked than with the seven-hour stage previously described, and may to some extent result also from the ingrowing connective tissue displacing certain of the epithelial cells from the positions which they had assumed after the discharge of the follicle. As a consequence of the ingrowth, the *membrana propria* no longer exists as an intact line. Wandering cells, free in the connective tissue, are found to occur not infrequently, but these do not appear to have been extravasated during the rupture of the vessels. In the case under consideration, practically no blood corpuscles are found remaining in the follicle's cavity or distributed among the epithelial cells, but in this instance, as in all other examples of recently ruptured Graafian follicles in the sheep that have come under my observation, a considerable quantity of blood was discharged to the exterior. A section through the wall of this follicle is figured on Plate 8 (fig. 8), where it may be compared with a section through the wall of an undischarged follicle (fig. 7).

It may here be noted that the vessels of ovaries containing recently discharged follicles may present signs of congestion apart from those in the region of the young corpus luteum. There is also evidence of increased blood supply to the ovaries during the proœstrum, *i.e.*, before the occurrence of ovulation, but I have never seen any breaking down of blood-vessels within the ovary, except in connection with a newly ruptured follicle.

In a discharged follicle obtained *sixteen hours* after coition (fig. 9, Plate 9) the connective tissue ingrowth is carried further, and cells, generally fusiform in shape and arising by mitotic division from those of the theca ingrowths, are beginning to penetrate between the epithelial cells. The increase of connective tissue is accompanied by a corresponding increase of vessels which take their origin from those of the theca wall, while the walls of vessels which had given way appear to have been recuperated. In this case also, extravasated blood corpuscles within the cavity of the follicle or free in the tissue are rare. The communication with the exterior where the follicle had ruptured is still widely open, and here red corpuscles and follicular epithelial cells are found in considerable quantities. The latter are either isolated or arranged in small groups, and already some of them present signs of degeneration, while many are slightly hypertrophied. Isolated epithelial cells presenting similar characters are numerous everywhere in the fluid-containing cavity, which is far smaller than that of the mature follicle. These cells had no doubt mostly formed part of the *discus proligerus*, but certain of them might have belonged to the *membrana granulosa*. The vast majority of the *granulosa* cells, however, still maintain their position, but have further increased in size. The average thickness of the epithelial wall of the corpus luteum at this stage is not shown in the figure, which was drawn at a point where this layer is much more reduced than elsewhere in the same follicle, the object being to represent a high-power magnification of a section through the complete follicular wall from the cavity to the *theca externa*.



The most remarkable characteristic of the corpus luteum of this stage of development is the number of leucocytes, which I have found to be far more abundant than at any other period. These leucocytes occur both in the connective tissue and also free in the follicular cavity. Many of them, at any rate, undergo degeneration, when they resemble degenerating follicular epithelial cells, which, as already noticed, frequently occur isolated in the cavity. In the case of the atretic follicle, degenerating epithelial cells have been mistaken for leucocytes, as remarked above (p. 72), while in certain later stages of the formation of the corpus luteum, I have found it difficult, if not impossible, to distinguish them when in a more advanced state of degeneration.

The corpus luteum of *twenty-four hours* has undergone further changes. Its increase in size is well marked, its dimensions now approaching those of the mature follicle. Its shape is irregular, while its walls have become more folded. The central cavity is smaller, but still communicates with the exterior, where it opens out into a cup-shaped depression on the surface of the ovary, from which the corpus luteum now appreciably protrudes. The epithelial wall of the cavity is at this period at least twice the thickness of that of the seven-hour stage, this increase being due for the most part to the simple hypertrophy of the individual cells composing it, these appearing in section to be at least two or three times the size of the membrana granulosa cells of the undischarged Graafian follicle. Division may, however, still be occasionally observed among the epithelial cells. But the thickness of this layer of the follicle's wall is also increased by the ingrowth of connective tissue, which by this time has become considerable. Groups of epithelial cells have become surrounded by a network of fusiform cells, derived from strands of ingrowing connective tissue, which give the young corpus luteum a radial appearance. The inter-epithelial connective tissue, consisting mainly of fusiform cells, and the hypertrophied epithelial cells are shown in fig. 10 (Plate 8), which represents a part of a section through the wall of a ruptured follicle of this stage. But although the connective tissue element of the corpus luteum of the sheep is provided chiefly by the proliferation by mitotic division of the cells of the theca interna, it is in part derived from the more fibrous theca externa, from which layer strands of cells, often in close connection with those of the inner layer, are at this period beginning to grow inwards among the epithelial cells. But the inner theca layer as such has to a great extent disappeared, having been used up in the formation of inter-epithelial connective tissue. It is to be noted, however, that the line of demarcation between the two thecae comprising the connective tissue wall of the follicle is not always well defined, either at the stage now being described or at other stages in the formation of the corpus luteum, while the boundary between the theca externa and the ovarian stroma is frequently still less definite, particularly in certain of the later stages. Extravasated blood corpuscles scattered in small patches and streaks occur in the twenty-four-hour stage, as in the earlier stages, but are not very numerous, there being no blood-clot within

the cavity. Leucocytes, though present, do not appear in such numbers as in the sixteen hour stage previously described.

In the corpus luteum *thirty hours* after coition the inner theca layer has all but vanished, while the ingrowth of strands from the outer theca is further advanced. The follicular epithelial cells are, on the average, slightly more hypertrophied, and the connective tissue network which in the previous stage had already begun to inclose some of them, has become more elaborated. The growth of connective tissue is accompanied by the increase in the number of blood-vessels. Red corpuscles extravasated at the time of discharge are more numerous within this follicle than in the cases of certain of the other stages, and are especially numerous in the proximity of the point of rupture, which is still open, and communicates with the fluid-containing cavity. Fig. 11 illustrates a section through the opening of the follicle, showing the depression on the surface of the ovary, and the beginning of the passage leading to the interior of the follicle. Islands of follicular epithelial cells are seen to be present near the aperture, and abundant extravasated blood corpuscles, as well as leucocytes; also newly formed blood-vessels, which have made their appearance with the ingrowth of the connective tissue, as shown in the figure. In some places I have found it a matter of great difficulty to distinguish between corpuscles extravasated at the time of the follicle's rupture, and corpuscles in thin-walled vessels which take their origin from those already existing in the theca wall. The bulk of the blood present, however, is clearly extravasated. Fig. 13 is a microphotograph of a transverse section of the ovary containing the ruptured follicle here described, and gives some idea of its size in relation to the rest of the ovary, besides showing the aperture of the follicle, and the communication with its already considerably obliterated cavity. (Plate 10.)

The epithelial cells of the corpus luteum of about *fifty hours* as seen in transverse section are four or five times the size of those of the undischarged follicle. Mitotic division is very rare among them, but evidence of it may still occasionally be observed. Proliferation of the connective tissue cells continues to take place, chiefly in the direction of the central cavity, which has become smaller. Leucocytes, some of which are apparently degenerate, are to be seen among the epithelial and fusiform cells, as well as free red corpuscles. A few degenerate epithelial cells also occur isolated in the cavity. The corpus luteum as a whole presents a radial appearance, which is emphasised by the ingrowth of large strands of connective tissue taking their origin from the theca externa. The inner theca layer, as such, has entirely disappeared.

The corpus luteum of *sixty hours* is larger than the ripe follicle. The epithelial cells are still increasing in size by simple hypertrophy, but I have not observed any case of division, the connective-tissue cells are dividing in all directions, so that nearly every epithelial cell has become surrounded by an anastomosis of fusiform cells. Blood-vessels, now of very considerable size, may be seen in many places in

the epithelium, in connection with the connective-tissue ingrowth, and especially near the periphery, where they are enclosed by large strands of cells arising from the theca externa. The point where the follicle had ruptured is still recognisable at the surface of the ovary, but the passage communicating with the interior is almost completely obliterated. The cavity itself is very much reduced, and, though still holding a fluid, no longer contains degenerating leucocytes or isolated epithelial cells, all traces of which seem to have entirely disappeared. Extravasated blood corpuscles, also, are absent in the specimen in question.

The process of obliteration of the cavity is effected, partly by the continued hypertrophy of the membrana granulosa cells and the increase of the inter-epithelial connective tissue, and partly by the formation of a wall of connective tissue within the follicular epithelium. This wall is formed of elongated cells arising from the continued ingrowth of the inter-epithelial connective tissue, and at the sixty-hour stage consists of a perfectly regular layer, about six cells deep, completely enclosing the cavity of the follicle. Fig. 12 illustrates part of a section through the wall of the follicle of this stage, and shows the inter-epithelial connective tissue, and the inner connective-tissue layer lining the edge of the now much obliterated cavity. (Plate 10.)

In the corpus luteum *three days* after œstrus, the process of formation has been carried still further. The point of rupture is only noticeable on the surface, where there is still a shallow depression, but there is no communication with the interior, the cavity having been all but filled-in by the further growth of connective tissue, the fluid which it contained being absorbed. The follicular epithelial cells as they appear in section are on the average about five or six times as large as those of the unaltered membrana granulosa from which they were derived, but their actual volume must obviously have increased by a much higher multiple. Fatty particles have already made their appearance in a very few of them which have begun to assume the character of true lutein cells. Meanwhile the inter-epithelial connective tissue has become still more finely distributed.

The discharged follicle *four days* after œstrus has already begun to assume the chief characteristics of the fully formed corpus luteum. The place where rupture had occurred has almost ceased to be visible, while the remains of the cavity are only to be found in a few sections passing through the centre. Fatty particles are somewhat more numerous in certain of the epithelial cells, which are approximately six times as large as the membrana granulosa cells, as they appear in section. The whole structure is a very little larger than the corpus luteum in the three-day stage of development. Extravasated blood corpuscles and wandering cells no longer occur, but the supply of blood-vessels is rich. The proliferation of connective tissue is still going on, especially in the form of ingrowing strands from the theca externa.

Certain further stages in the formation of the corpus luteum, the ages of which were not known, show a more advanced degree of development. The dimensions

of these were greater than with the stages described above, and were considerably in excess of those of the ripe Graafian follicle. The size of the corpus luteum is, however, no sure guide to its age, for I have observed two in the same ovary and of the same degree of structural development but of slightly different dimensions. In the more advanced stages, the epithelial cells continue to hypertrophy until, as seen in section, they have attained more than six times their original size. Meanwhile the number of fatty particles in them has increased, and they have become converted into those lutein cells which characterise the fully developed corpus luteum.

*Other accounts of the Formation of the Corpus Luteum.*—The first to discuss the mode of development of the corpus luteum was VON BAER (1827), according to whom the structure resulted from changes in the connective-tissue wall of the follicle, the membrana granulosa not participating in the process. Subsequently PATERSON (1840), who described the corpus luteum of the sheep among other animals, attributed its formation to changes in the blood coagulum left within the cavity of the follicle after its discharge. About the same time, BISCHOFF (1842) adopted the view that the lutein cells were derived from the membrana granulosa. PATERSON'S hypothesis never gained any general support, but the theory of VON BAER, which supposes the corpus luteum to be an entirely connective-tissue structure, and that of BISCHOFF, deriving the lutein cells from the follicular epithelium, have, with comparatively slight modifications, gained numerous adherents.

To SOBOTTA belongs the credit of being the first to adequately apply the experimental method to the solution of the problem, and with the publication of his investigation, the controversy regarding the mode of formation of the corpus luteum entered upon a new phase. I do not propose to cite afresh the numerous authors who have written upon this question prior to the publication of SOBOTTA'S first paper (1895) on the corpus luteum of the mouse, since an admirable résumé of the earlier as well as the later literature of the subject has been given by SOBOTTA himself in MERKEL and BONNET'S 'Ergebnisse der Anatomie und Entwicklungsgeschichte,' (1899), to which the reader is referred.

SBOTTA, in obtaining the developmental stages of the corpus luteum of the mouse (1895, 1896), and the rabbit (1897), collected his material exclusively from animals kept under observation, and killed at stated periods after coition, so that SOBOTTA adopted the same experimental method as that employed by the present writer in investigating the corpus luteum of the sheep. His researches resulted in confirming BISCHOFF'S hypothesis that the lutein cells are derived from the follicular epithelium, while the inner theca wall of the follicle is shown by SOBOTTA to give rise only to an inter-epithelial network, and to a part of the connective tissue, which fills in the cavity of the ruptured follicle.

Since the result of my investigation on the corpus luteum of the sheep agrees essentially with that obtained by SOBOTTA for the corpora lutea of the mouse (1896) and the rabbit (1897), it will be convenient to refer merely to the more important

differences between his account and mine. Some of these differences have been mentioned by SOBOTTA in his most recent paper (1902) while commenting on my 'Preliminary Communication' (1901).

According to SOBOTTA's descriptions, the theca externa takes no part in the formation of the corpus luteum either in the mouse or in the rabbit, the connective-tissue proliferation being derived entirely from the theca interna, in this respect differing from the corpus luteum of the sheep. But I am able to confirm SOBOTTA's statement in the case of the mouse, for in sections through corpora lutea belonging to certain of the more advanced stages, kindly lent me by Dr. J. H. ASHWORTH, I have been unable to recognise any ingrowth from the theca externa. The nature of the connective-tissue proliferation in the early stages also differs somewhat in the two accounts, as a comparison between my figures and those of SOBOTTA will show, the ingrowths from the theca interna arising generally as bud-like processes in the case of the sheep's corpus luteum. Another point of difference relates to the mode of obliteration of the central cavity, which according to SOBOTTA is in part filled in by the transformation of leucocytes into star-shaped connective-tissue cells, and not merely by the further ingrowth of connective tissue arising from the proliferation of the cells of the theca wall. In the development of the sheep's corpus luteum leucocytes are present in great numbers in the early stages, and especially in the sixteen-hour stage, as described above, but many of them are in a degenerate condition, while in subsequent stages, prior to the filling of the cavity, leucocytes are almost entirely absent. In the sixty-hour stage, the process of filling-in of the cavity has started, and is seen to occur by the formation of a regular wall of cells, arising from the inter-epithelial connective tissue. (Fig. 12, Plate 10.)

SBOTTA describes the follicular epithelial cells in the mouse and rabbit, as becoming transformed into lutein cells by a process of simple hypertrophy, mitotic division almost entirely ceasing after the follicle's rupture. My own observations on the sheep's corpus luteum to a very great extent confirm SOBOTTA's account, but I have shown that mitotic division among the epithelium may continue with decreased frequency in the earlier stages.\*

Other differences between SOBOTTA's account and mine relate to the absence, in every case, of a blood coagulum within the sheep's ruptured follicle, and to the fluid found in the cavity of the latter, this varying somewhat in quantity, and resembling the liquor of the undischarged follicle. SOBOTTA shows that the place where the follicle ruptures heals up very rapidly in the case of the mouse, while in the rabbit, as in the sheep, it remains open for a considerable period. The tendency to greater irregularity in shape with the developing the corpus luteum of the sheep, as compared especially with the mouse, though noticeable, is a point of no significance.

The development of the corpus luteum in the rabbit has been investigated also by

\* SOBOTTA (1897) describes a single instance of mitotic division in a follicular epithelial cell of a young corpus luteum of a rabbit.

HONORÉ (1900), who obtained a series of stages similar to those procured by SOBOTTA, the animals being killed at intervals after copulation, as in SOBOTTA'S researches. HONORÉ'S conclusions are almost in entire accord with those of SOBOTTA, but the more recent investigator describes the theca externa as taking part in the growth of the inter-epithelial connective tissue, in this point differing from SOBOTTA, but agreeing with my observations on the formation of the corpus luteum in the sheep. On the other hand, according to HONORÉ, the whole of the theca interna is not used up in giving rise to the connective-tissue anastomosis, whereas according to the investigations of SOBOTTA and myself this layer entirely disappears in comparatively early stages of development.

The observations of STRATZ (1898) upon developing corpora lutea in *Tarsius*, *Tupaia*, and *Sorex* are in general agreement with SOBOTTA'S descriptions. VAN DER STRICHT'S account (1901) of the formation of the corpus luteum in bats (*Vesperugo*, *Plecotus*, and *Vespertilio*), however, presents differences of some importance. This author agrees with SOBOTTA in describing the follicular epithelial cells as persisting and giving rise to the lutein cells, but he adopts the view that all the lutein cells are not formed in this way, a few being derived from interstitial cells of the theca interna. VAN DER STRICHT also differs from SOBOTTA in describing the not infrequent occurrence of mitotic division, in addition to simple hypertrophy, among the lutein cells, and notes such division in the developing human corpus luteum, as well as in that of the bat. In other respects VAN DER STRICHT'S description is confirmatory of that of SOBOTTA. A special point of interest in VAN DER STRICHT'S account is the very early appearance of fatty particles in the follicular epithelial cells of the bat's corpus luteum.

KOPSCH, at the meeting of the "Anatomische Gesellschaft" at Bonn, exhibited some preparations of corpora lutea belonging to three stages of development (three, six, and ten-day stages) from the sow, confirming the results of SOBOTTA, HONORÉ, STRATZ, and the present writer regarding the origin of the lutein cells (SOBOTTA, 1902). SOBOTTA (1899) states also that BONNET has to some extent investigated the formation of the corpus luteum in the dog, and obtained similar results.

KREIS (1899), writing on the development of the "corpus luteum spurium"\* or "corpus luteum menstruationis," which he appears to regard as distinct both from the corpus luteum verum (*i.e.*, the corpus luteum of pregnancy) and from the atretic follicle, describes the epithelium as being retained in the follicle, but adds nothing to the discussion on the origin of the lutein cells.

BELLOY'S account (1899) of the formation of the corpus luteum in the rat and the cavy differs from all others in describing an active multiplication of the follicular epithelial cells, which he seems to me to have confused with the ingrowing

\* The corpus luteum of pregnancy, or corpus luteum verum, and the corpus luteum spurium or menstruationis are, by derivation, necessarily identical structures, and are generally so regarded.

connective-tissue cells. At any rate, BELLOY does not appear to recognise the nature of the connective-tissue proliferation.

Mr. HEAPE (1897), without discussing the origin of the lutein cells, states that the increase in the size of the wall of the discharged follicle in monkeys is due to the hypertrophy of its cells, and not to an increase in their number, this result having been arrived at quite independently of SOBOTTA's research.

RABL (1898), who has also written on the formation of the corpus luteum, is of opinion that the lutein cells arise partly from the follicular epithelium and partly from the connective tissue wall.

The authors cited above have all to a greater or less extent confirmed SOBOTTA's account of the formation of the corpus luteum. A number of investigators, however, since the publication of SOBOTTA's work have adopted the directly opposite hypothesis, that the corpus luteum is an exclusively connective-tissue structure. Among them KOELLIKER (1898) and HIS (KOELLIKER, 1898), at the meeting of the "Anatomische Gesellschaft" at Kiel, made statements upholding their previously formed conclusions, while NAGEL (1896, see also NAGEL, 1899) has also described the lutein cells as arising from the connective tissue of the theca wall. The same opinion is likewise maintained in a recent discussion of the question by PALADINO (1900).

CLARK (1898), after investigating the formation of the corpus luteum in the sow and in the human subject, has convinced himself of its entirely connective-tissue origin, expressing the hope that he had obtained results which could leave no doubt regarding its mode of development; while DOERING (1899), also writing on the corpus luteum of the sow, claims to have confirmed CLARK's account. Others who have recently taken the same view are BÜHLER (1900), WENDELER, and STÖCKEL, the last author stating that he is forced to the conclusion that the lutein cells are derived from the theca interna.

The descriptions of all these authors have been dealt with by SOBOTTA (1898, 1899, 1902), so that it is unnecessary for me to do more than to refer to their papers, and to state that I am substantially in agreement with SOBOTTA's criticisms. The results arrived at by NAGEL, CLARK, and those who uphold the connective-tissue origin of the corpus luteum, are due largely to confusion between discharged and atretic follicles, and between the young and somewhat later stages in the development of the discharged follicle, this confusion resulting in great measure from the absence of method in procuring material.

As already shown, my own account of the sheep's corpus luteum agrees essentially with that given by SOBOTTA for the mouse and the rabbit, and by HONORÉ for the rabbit, the material in each investigation having been obtained according to the experimental method. In so largely confirming their accounts, my results, as SOBOTTA (1902) points out, in commenting on my preliminary paper (1901), indicate the falsity of the suggestion of HIS that the mode of development of the corpus luteum in the larger mammals radically differs from what occurs in the mouse and the rabbit.

SOBOTTA alludes also to the rapidity of the process of formation of the sheep's corpus luteum, a rapidity which is all the more remarkable owing to its resulting, to so great an extent, from the simple hypertrophy of certain of its constituent cells. A parallel to this rapid cellular hypertrophy may perhaps be found in the development of the uterine outgrowth in the Nematode *Sphærulearia*, the cells of which are described (SHIPLEY, 1896) as increasing in size but not in number, as is so largely the case with those of the follicular epithelium, which plays such an important part in the formation of the corpus luteum.

#### *Summary and General Conclusions.*

In Scotch Black-faced sheep the duration of the diœstrous cycle varies from about two to three weeks, according to the locality, and the same is probably the case with sheep belonging to other breeds. The length of the sexual season and the number of recurrent diœstrous cycles in a single season are also subject to variation, there being a perfect gradation from the diœstrous condition of the Highland Black-faced sheep to the extreme of polyœstrum reached by certain Merinos. The difference is in part related to the food supply and environment, but increased sexual power is also a racial characteristic, as is shown, for example, by the Dorset Horn and Limestone sheep, and by the sheep of Patani. Wild sheep are probably for the most part monœstrous, with a tendency to polyœstrum under favourable conditions. The same may perhaps be said for ruminants generally, but from want of evidence it is still impossible to form any conclusion in regard to whether monœstrum or polyœstrum is the primitive condition for the Mammalia as a whole.

The changes through which the sheep's uterus passes during a single diœstrous cycle can be divided into four groups or periods, which are as follows:—(1) Period of rest; (2) period of growth and increase of vessels; (3) period of breaking down of vessels and extravasation of blood; (4) period of recuperation and pigment formation. These headings indicate the nature of the changes which the uterus undergoes. Bleeding into the uterine cavity and through the external generative opening need not necessarily occur. The extravasated blood left in the uterine stroma forms pigment, which consequently tends to accumulate as the sexual season advances. But the amount of pigment formed depends also on the degree of severity of the proœstrous changes. Where these processes are more severe, there is less extravasated blood left behind in the stroma, and so less to take part in pigment formation. Extravasation takes place almost entirely in the more superficial portion of the stroma, the vessels in the deeper part of the uterus remaining intact. There is also some evidence that the severity of the proœstrous process tends to diminish with each successive diœstrous cycle in one sexual season. There is never any denudation of stroma, and the uterine epithelium disappears only in places where bleeding into the uterine cavity occurs. The pigment formed during the sexual season must



eventually be removed, since I have never found it present in ewes killed during the aœstrous period.

That the dicestrous cycle of the sheep and the menstrual cycle in the Primates are physiologically homologous is rendered extremely probable in the light of the facts summarised above, while if a further comparison be made with other Mammalia, and more particularly the dog and the ferret, the probability becomes converted into a certainty. The proœstrum in the ferret, according to my own researches (an advanced abstract of which is embodied in the present memoir), in regard to its severity is almost intermediate between that of the sheep on the one hand, and the menstruation of the Primates on the other.

œstrus in the domestic sheep very rapidly succeeds the proœstrum, so much so that the period of desire seems sometimes to be coincident with the proœstrum. But this is where the proœstrous process is less severe. When external bleeding does occur in ruminants, as in bitches and ferrets, there is evidence that it takes place prior to the actual œstrus period, this being known to be the case with certain heifers.

Since the proœstrum and menstruation are homologous, and since in at least many Mammalia, coition and the fertilization of the ovum (if not ovulation) occur after the proœstrum, it follows that the changes characterising the degeneration and denudation stages of the proœstrum and menstruation are not of the nature of an undoing, owing to the absence of a fertilized ovum, of preparations made during the growth period. But the alternative view, that the whole proœstrous process (of which the changing of the uterine tissue is a not unimportant part) is in a general way an act of preparation, appears to me to be perfectly tenable. On this hypothesis, it does not follow that the process must be directly associated with any particular ovulation.

With the sheep, ovulation takes place during the sexual season; it can occur during any œstrus (or possibly occasionally during a proœstrum) in the regular sexual season with Scotch Black-faced ewes without the additional stimulus supplied by sexual intercourse. But it would seem that in œstrus periods, which, for one reason or another, exist outside the regular sexual season of the particular breed, the extra stimulus furnished by coition and the presence of sperms in the uterus, may be necessary before ovulation can be induced. There is evidence also that coition during any œstrus period may hasten forward the ovulation, if it has not already occurred, at that period.

The development of the corpus luteum (the experimental study of which necessarily resulted from the investigation on the periods when the ova ripen) in the sheep was found to resemble that of the mouse and rabbit, as described by SOBOTTA, in its essential features, the lutein cells being the much-hypertrophied epithelial cells of the undischarged follicle. But the connective tissue element of the corpus luteum in the case of the sheep is supplied not only by ingrowth from the inner theca, but also by

the theca externa. The cavity of the corpus luteum becomes filled up by connective tissue derived similarly from the follicular thecæ, and is not formed from leucocytes. But in some of the earlier stages leucocytes are very abundant, wandering inwards to the cavity with the ingrowth of the connective tissue. Later they appear to undergo degeneration, and take no part in the formation of the corpus luteum.

The atretic follicle can be readily distinguished from the discharged follicle or young corpus luteum by the absence of any indication of a point of rupture, the degeneration of the epithelial cells, and the character of the connective-tissue ingrowth, which does not occur to an appreciable extent until the epithelium is in an advanced state of degeneration, or has entirely disappeared. The retention of a degenerate ovum within the cavity is also an almost sure indication of an atretic follicle.

Scattered red corpuscles may be present in the cavity both in an atretic follicle and also in a young corpus luteum in the case of the sheep.

I found atresia to set in most commonly among follicles that had reached from about one-half to one-eighth the size of the ripe follicles, but it may occur at any time. When taking place to any considerable extent, it must inevitably raise the barrenness percentage, so that the subject of atresia is not without its practical interest. In Scotch Black-faced sheep (whether pure-bred or half-bred) I found that the number of follicles discharged at a single ovulation only exceeded two in one instance, and that it was far more often one than two. So that evidence points to the conclusion that the number of follicles discharged at an ovulation is, on an average, scarcely in excess of the number of lambs produced at a time. From this it may be inferred that scarcity of twins, or even complete barrenness (whether temporary or otherwise) results from failure on the part of the ovary, probably owing to an excess of follicular atresia, whether occurring during the sexual season or at some previous period.

I have no evidence that œstrus can occur in ewes in the total absence of mature follicles in both ovaries, but I see no reason even in such animals as the sheep, for regarding the connection between ovulation and œstrus to be such an essentially close one as is often maintained. According to all the evidence of sheep-breeders, temporary barrenness is not necessarily associated with omission of œstrus (or extension of anœstrum), and I found no relation between the intensity of the œstrous (and proœstrous) phenomena and the number of follicles discharged during the particular œstrus.

Mr. HEAPE (1900) has laid some stress on the fact that ovulation is not always associated with menstruation in the Primates, and that it may require a stimulus in addition to that supplied by œstrus in the case of the female rabbit; and in the present paper I have shown that with the bitch ferret while the proœstrum and œstrus are distinct periods, the latter may continue for several weeks, during which the animal will permit the occurrence of coition, although the time for ovulation has passed by.

It would appear then that while the rate of growth and maturation of the follicles are the result of nutriment and general physical condition, besides being dependent partly upon seasonal changes, the actual rupture of these follicles and the discharge of the ova can, in the sheep and many other animals (though certainly not in all), only take place as a result of a special stimulus. In this sense œstrus, and in some instances sexual intercourse, may be said to determine ovulation, but not the periods when ova ripen. But the uterine changes characterising the diœstrous cycle are themselves in part also the result of nutritive and seasonal influences. So that the phenomena of ovulation and œstrus are connected, though not always necessarily interdependent.

The relation between the development of the corpus luteum and the changes occurring in the uterus during pregnancy is a closer one. According to BORN the corpus luteum is of the nature of a gland, the function of which is to secrete into the blood substances which in a general way prepare the uterus for pregnancy.\* This view is supported by FRAENKEL and COHN (1901), who have made experiments with rabbits, showing that when the corpora lutea are destroyed by a galvano-caustic needle, pregnancy cannot continue. Whether BORN'S theory is right or wrong, FRAENKEL and COHN have undoubtedly demonstrated a very intimate connection, already known to be close, between the existence of the corpus luteum and the occurrence of pregnancy in so far as the latter appears to be in some way dependent upon the former. But on the other hand, it is a now generally accepted fact that whereas the ovarian changes characterising the early development of the corpus luteum are the same, whether or not the discharged ovum is to become fertilised (in other words, that the so-called corpora lutea vera and spuria are by origin identical structures), yet the later changes depend upon the occurrence or non-occurrence of pregnancy. In this sense the further development or degeneration of the corpus luteum may be said to be determined by the state of the uterus.

From these and similar facts it may be inferred that the functions of ovulation and œstrus do not stand to one another respectively either in the relation of cause to

\* The questions concerning the ovary generally as an organ of internal secretion, and its possible functions apart from the production of ova, have less bearing on the subject of the present memoir. For a recent summary of the work done on these questions, DIXON'S paper (1901) may be consulted. It is stated that after the performance of ovariectomy, menstruation generally ceases, the small percentage of cases where it still continues being accounted for on the supposition that both of the ovaries were not completely removed. But ovariectomy, like castration with male animals, has a profound influence over the constitution as a whole, often seriously impairing the health, and not merely preventing the occurrence of menstruation and œstrus. GLASS has shown that transplantation of the ovary, after ovariectomy had been previously performed and had induced distressing symptoms and cessation of menstruation, may result in restoration to health and renewal of menstruation (DIXON, 1901). The statements of FERRÉ and BESTION (DIXON, 1901), based on work at present unconfirmed, that injection of ovarian extract may induce genital excitement, more directly concern the subject of this paper, but it is to be noted that unhealthy symptoms, paralysis, &c., were also brought about by the injections. (See also HEAPE, 1898, and HALBAN, 1901. In the latter paper the effects of ovariectomy with monkeys are described.)

effect or conversely, but are connected primarily, inasmuch as each is dependent largely upon the same causes, while a further connection, having regard especially to the changes relating to pregnancy, has become established between them, the two sets of functions acting and reacting upon each other; and that whereas this interrelation in the majority of the Mammalia is closely maintained, in certain cases, as with the ferret, and more evidently with the Primates, the factors which control the œstrous cycle act to a varying extent independently of those which govern ovulation.

In conclusion, I wish to thank all those who have assisted me in my investigations. To Professor EWART, in connection with whose "Biological Farm" at Penycuik, and whose laboratory in the University of Edinburgh the work was carried out, I am especially indebted for the numerous facilities he has placed at my disposal and for the encouragement he has always given. My best thanks are due also to Mr. WALTER HEAPE for giving me the benefit of valuable suggestions and information, and for the sympathetic interest he has shown, both at the commencement and throughout the progress of my work. To Professor SCHÄFER I am not a little indebted for kindly encouragement and help, more especially with the histological part of the work. Lastly, I take this opportunity of recording my great indebtedness to Sir THOMAS GIBSON CARMICHAEL, Bart., for the support he has so generously given.

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## DESCRIPTION OF THE PLATES.

## PLATE 7.

Transverse sections through the uterine cornua, showing portions of the uterine wall,  $\times 220$ .

*Reference Letters.*

*b. v.*, blood-vessel; *b.v.rup.*, recently ruptured blood-vessel; *c. t.*, connective tissue of the stroma; *ep.*, epithelium lining uterine cavity; *ep. gl.*, epithelium of gland; *ex. b.*, extravasated blood corpuscles; *ex. b. p.*, ditto in process of transformation into pigment; *f.*, muscle fibres; *lac. rup.*, ruptured lacuna; *leu.*, leucocyte; *p.*, pigment; *p.c.*, pigment cell.

Fig. 1.—Section showing a portion of the mucosa of an intercotyledonary area, containing a few muscle fibres. (Period I.)

Fig. 2.—Section showing a portion of the mucosa of a cotyledon. (Period II.) The stroma nuclei are most thickly distributed in the proximity of the epithelium.

Fig. 3. Section showing a portion of the mucosa of a cotyledon. (Period III.)

Fig. 4. Section showing a portion of the mucosa of a cotyledon. (Period III, advanced stage.)

Fig. 5. Section showing a portion of the mucosa of a cotyledon at an early stage of pigment formation.

Fig. 6. Section showing a portion of the mucosa of a cotyledon during a stage of pigment formation. Large quantities of completely-formed pigment lying apparently free in the stroma are also seen.



## PLATES 8-10.

Transverse sections through ovaries, showing portions of the walls of follicles or developing corpora lutea.

*Reference Letters.*

*bl. v.*, blood-vessel; *cav.*, cavity. (The fluid in the cavity is represented only in fig. 9.) *c. t.*, connective tissue; *dep.*, cup-shaped depression on surface of ovary at point of rupture of follicle (containing islands of epithelial cells, as well as isolated epithelial cells); *ep.*, follicular epithelium or "membrana granulosa"; *ep. c.*, follicular epithelial cell; *ex. b.*, extravasated blood; *fus. c.*, fusiform cell; *leu.*, leucocyte; *pas.*, passage leading from depression (representing point of rupture) to internal cavity; *th. int.*, theca interna; *th. ext.*, theca externa.

## PLATE 8.

- Fig. 7. Section through wall of Graafian follicle  $\times 300$ .  
 Fig. 8. Section through wall of discharged follicle (seven-hour stage)  $\times 300$ . The ingrowth of connective tissue from the theca interna and the hypertrophy of the follicular epithelial cells have commenced.  
 Fig. 10. Section showing part of wall of discharged follicle (twenty-four-hour stage)  $\times 300$ . The connective-tissue proliferation has become considerable.  
 Fig. 11. Section showing part of wall of discharged follicle (thirty-hour stage)  $\times 100$ .

## PLATE 9.

- Fig. 9. Section through wall of discharged follicle (sixteen-hour stage)  $\times 300$ .

## PLATE 10.

- Fig. 12. Section showing inner part of wall of discharged follicle (sixty-hour stage)  $\times 100$ .  
 Fig. 13. Microphotograph of transverse section through ovary containing a discharged follicle (thirty-hour stage) showing place of rupture and ingrowths in the form of strands arising from the theca externa,  $\times 16$ .

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[Figs. 1-6 were drawn by Mr. J. C. BRODIE, of Edinburgh.

Figs. 7-12 and also the microphotograph (fig. 13) were the work of Mr. RICHARD MUIR, of Edinburgh. The clearness and accuracy of Mr. MUIR's original drawings are, unfortunately, to some extent lost in the reproductions. This is seen especially in the absence of outline in the walls of certain of the blood-vessels.—June 2nd.]



Fig 1

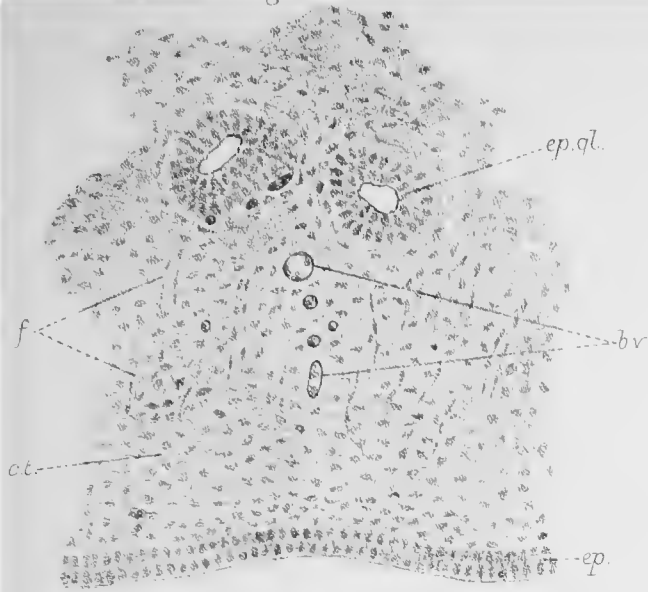


Fig 4.

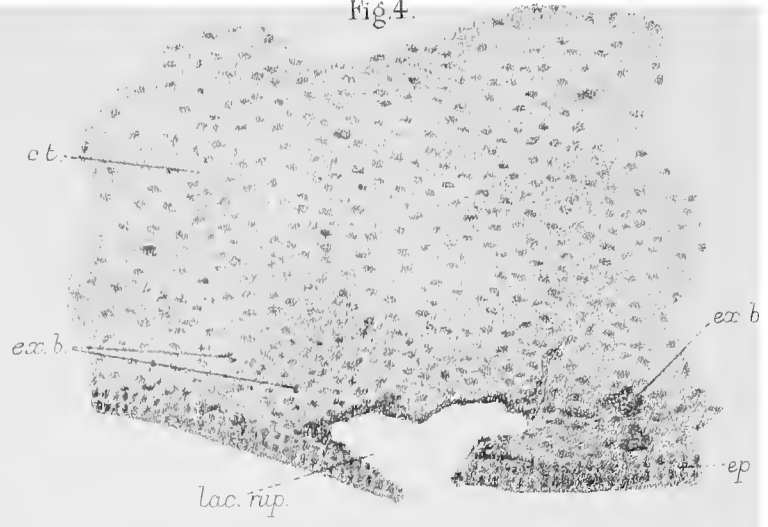


Fig 2

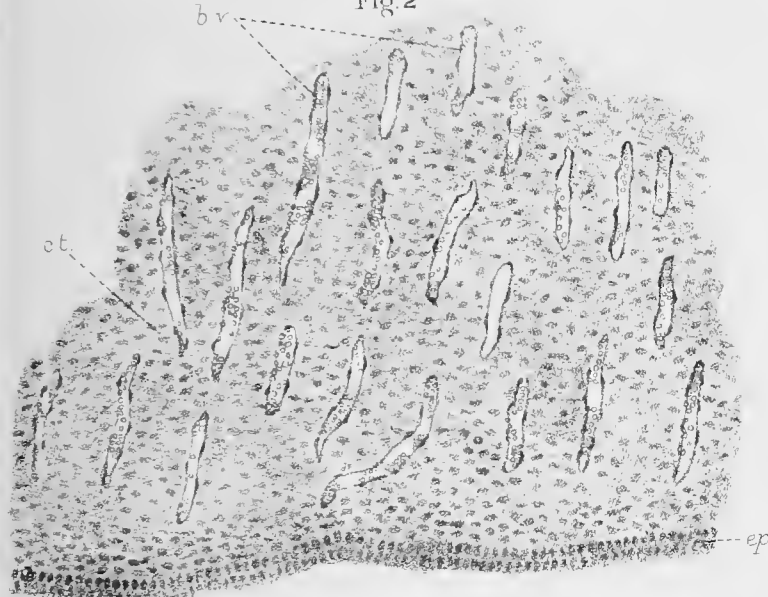


Fig 5.

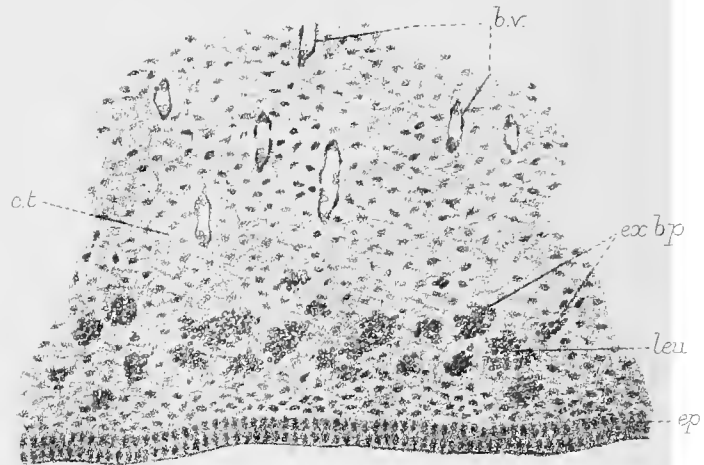


Fig 3



Fig 6.

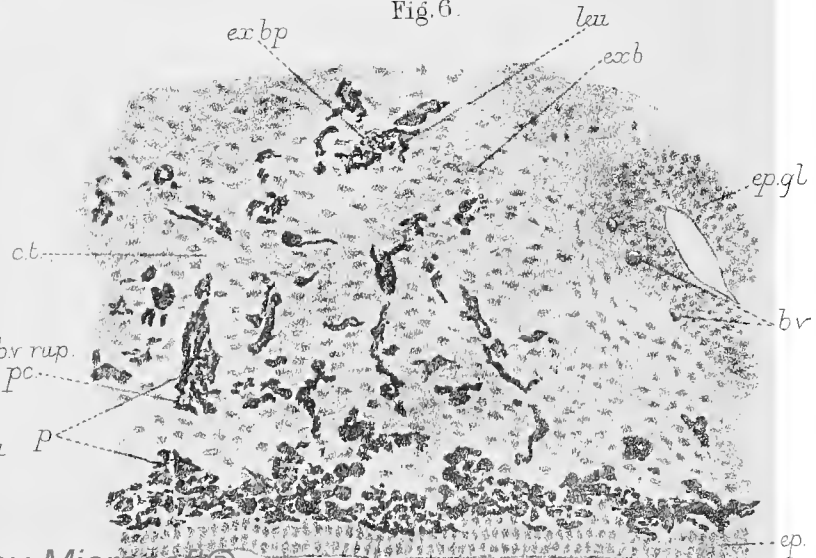




Fig 7

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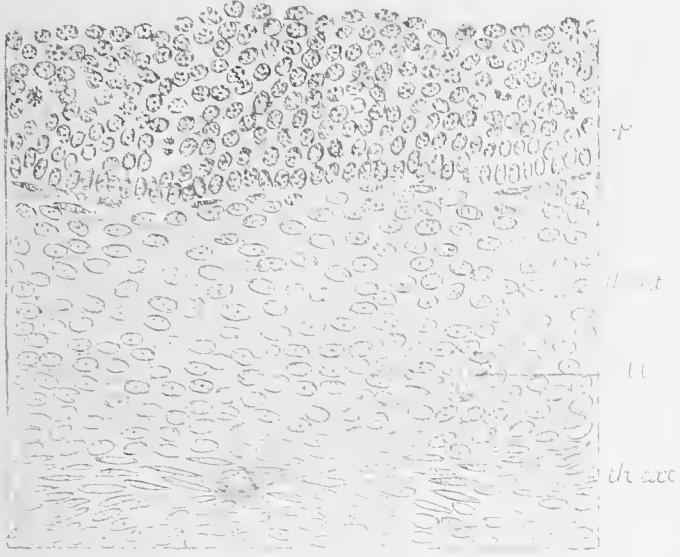


Fig 8

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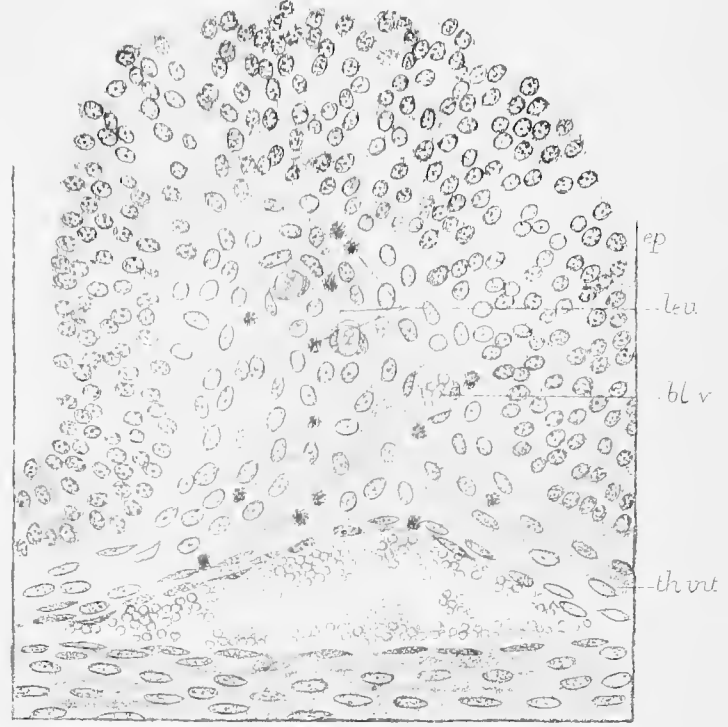


Fig 11

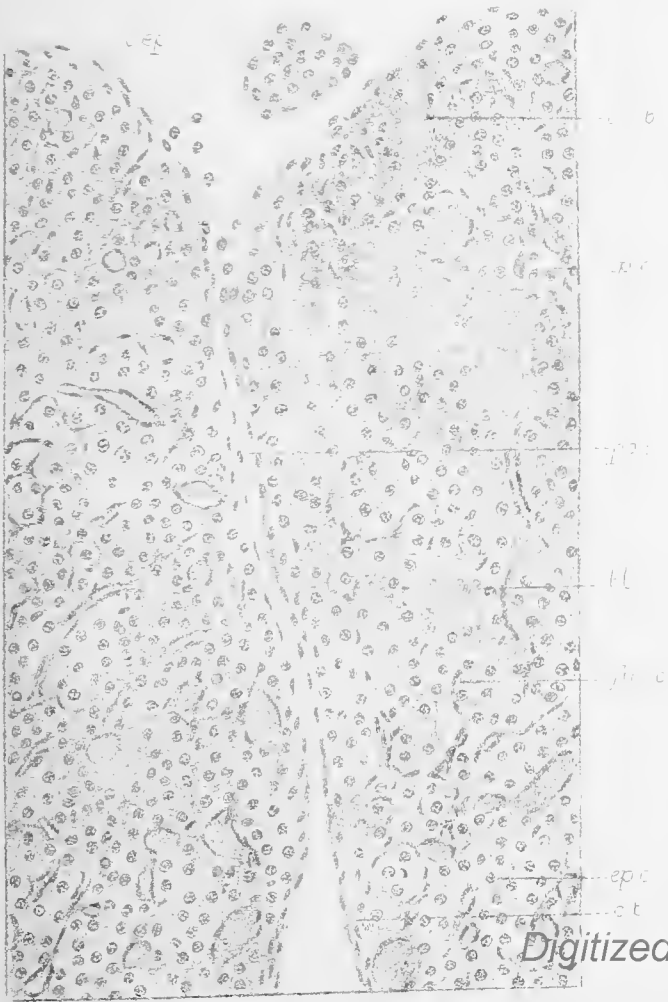
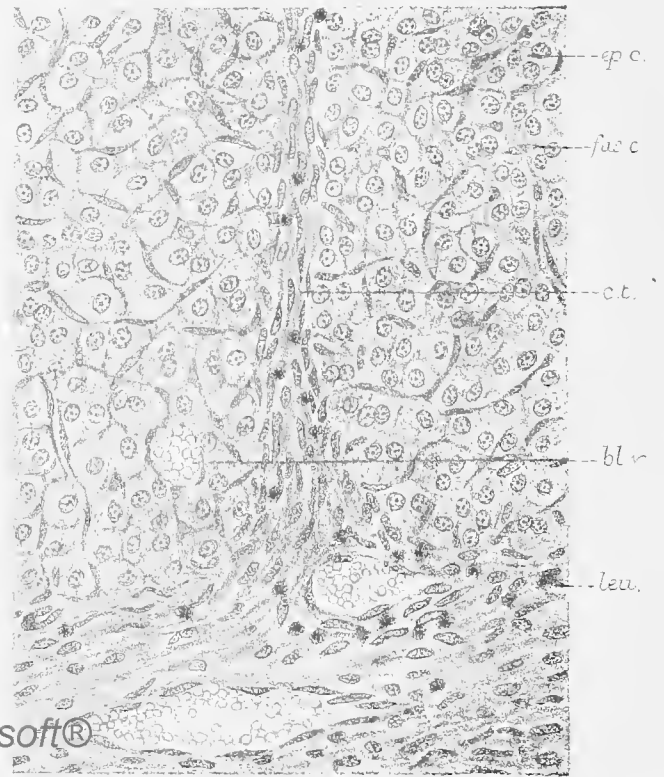
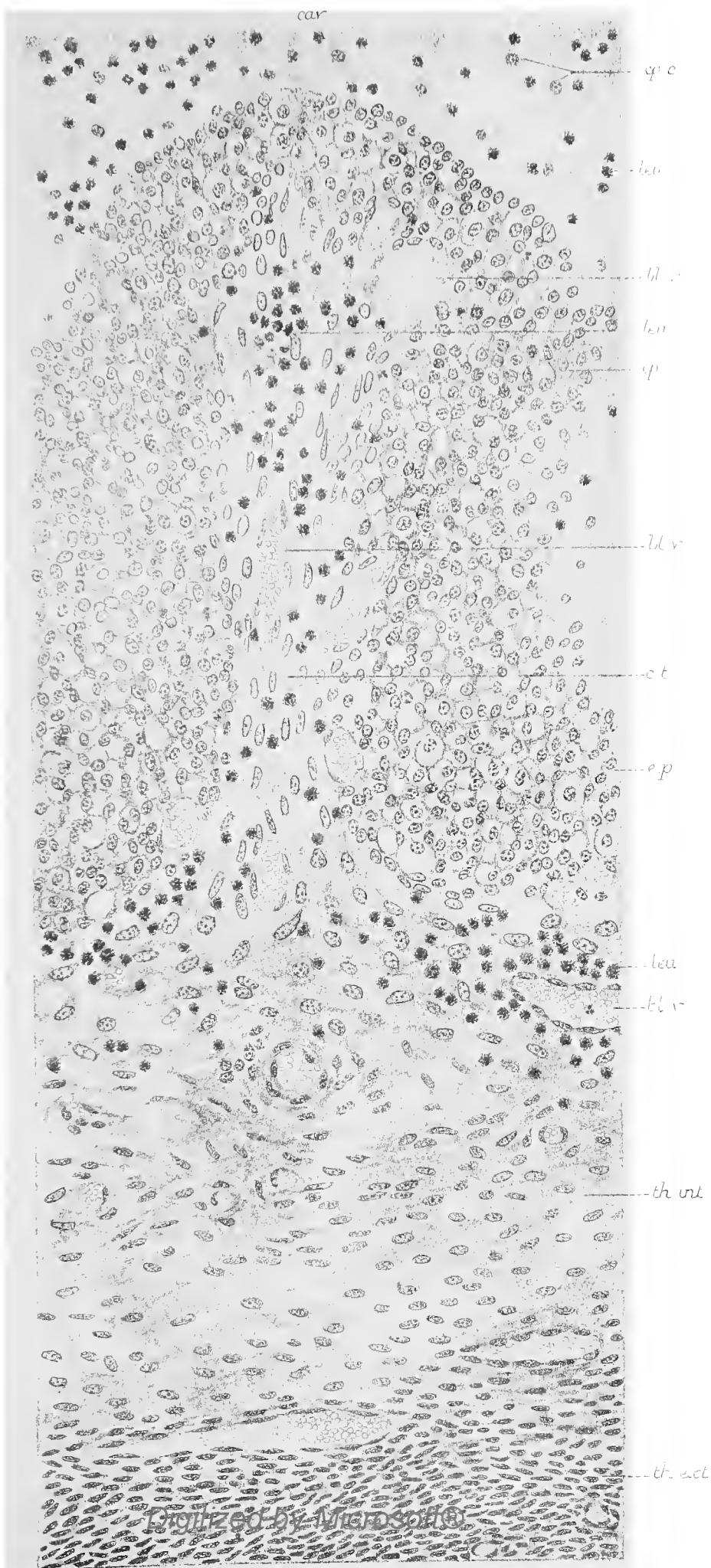


Fig 10







Engraved by Microspite





Fig 12.

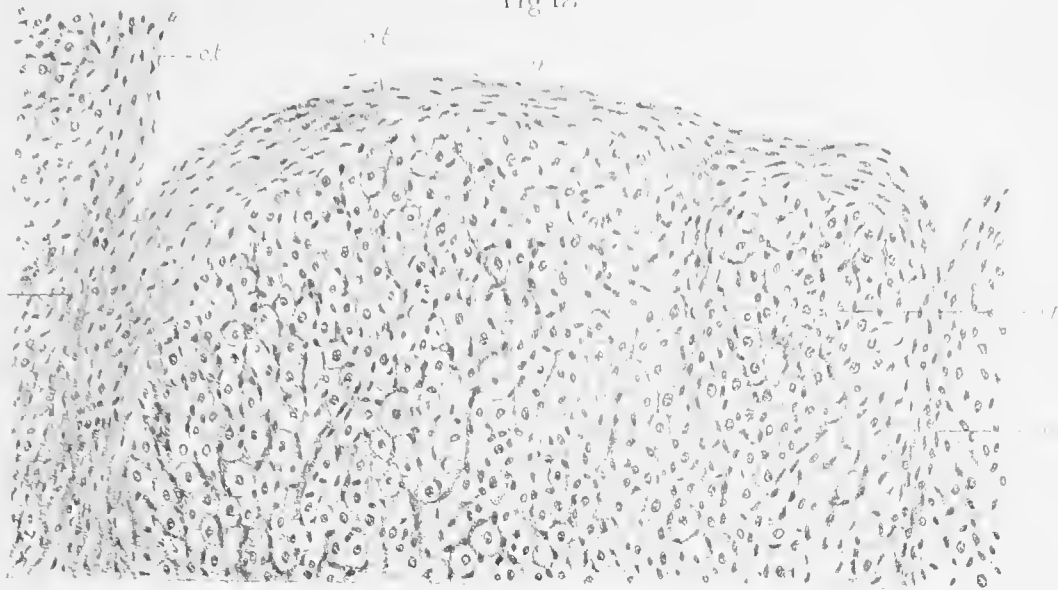
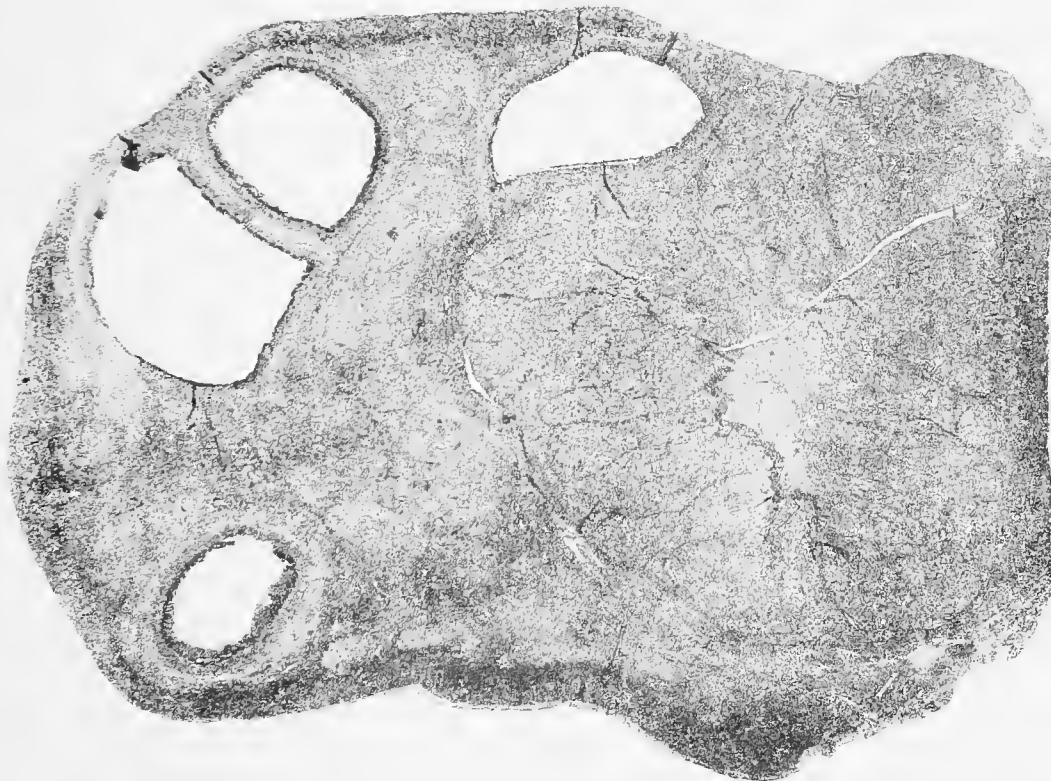


Fig 13.





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